**THE EFFECTS OF SKELETAL SEPARATION AND MOISTURE
ENHANCEMENT ON IMPROVING THE EATING QUALITY OF CULL COW
BEEF**

A Thesis

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of

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by

PETER JOSEF STREITER

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ABSTRACT

THE EFFECTS OF SKELETAL SEPARATION AND MOISTURE ENHANCEMENT ON EATING QUALITY OF CULL COW BEEF

Peter Josef Streiter
University of Guelph, 2010

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Dr. Ira B. Mandell

Cull cow beef is known to be inconsistent in tenderness and juiciness and it could benefit from postmortem processing. Sixty-two cull beef cows of known age and breed were slaughtered to investigate the effects of skeletal separation and moisture enhancement effects on eating quality. Four postmortem processing treatments included: 1) No postmortem processing (NPP), 2) Prerigor skeletal separation (SS), 3) Moisture Enhancement (ME) using Calcium Ascorbate or 4) Combination SS and ME (SS/ME). The muscles investigated in this study included *longissimus* muscle from the ribeye and loin, *semimembranosus* and *semitendinosus*. SS increased sarcomere length in the *Longissimus* muscle. SS and ME decreased Warner-Bratzler shear force in the *Longissimus* muscle. SS and ME increased *Longissimus* tenderness and juiciness versus NPP. An additive effect exists when combined SS and ME improved palatability traits in cull cow beef compared to either SS or ME alone. Postmortem processing of beef cows can produce beef as tender and juicy as Canadian AA and AAA marbled beef.

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LIST OF ABBREVIATIONS

A	Age at slaughter in years
AA	AA marbled beef (USDA Select equivalence): one of Canadian Quality Grades for youthful carcasses
AAA	AAA marbled beef (USDA Choice equivalence): one of Canadian Quality Grades for youthful carcasses
APC	Aerobic plate counts
BHT	Butylated hydroxytoluene
BRIT	Animals with predominantly British breeding
CaAsc	Calcium Ascorbate
CaCl₂	Calcium Chloride
cfu	Colony forming units
CONT	Animals with predominantly Continental Breeding
DFD	Dark, firm, dry
DOSL	Date of Slaughter
EDTA	Ethylenediaminetetraacetic acid
G	Grade fat
HCl	Hydrochloric Acid
LHS	Left hand side
LM	<i>Longissimus</i> Muscle
LMA	<i>Longissimus</i> Muscle Area
LTL	<i>Longissimus</i> Thoracis et Lumborum
MDA	Malondialdehyde

ME	Moisture Enhancement
MOLAS	Modified On-Line Altered Suspension
NA	Not Applicable
NaCl	Sodium Chloride
NPP	No Postmortem Processing Treatment
NPPC	National Pork Producers Council
PO₄	Phosphate
PPT	Postmortem Processing Technique
PVC	Polyvinyl Chloride
RE	Rib Eye
RHS	Right hand side
SF	Shear force
SM	<i>Semimembranosus</i> Muscle
SS	Skeletal Separation
SS/ME	Skeletal Separation and Moisture Enhancement
ST	<i>Semitendinosus</i>
LOIN	Strip Loin (<i>Longissimus</i> section)
TBARS	Thiobarbituric Acid
WBSF	Warner-Bratzler Shear Force
WHC	Water holding capacity
wt	Weight
<i>Physical units</i>	
°C	Degrees Celsius

cm	Centimetre
g	Gram
kg	Kilogram
l	Litre
M	Molar
mg	Milligram
ml	Millilitre
mm	Millimetre
N	Normal
%	Percent
rpm	Revolutions per minute
μl	Microlitre
μm	Micrometre
μM	Micromole
<i>Units of time</i>	
d	Day
h	Hour
min	Minute
mo	Months
s	Second
yr	Year
wk	Week

Statistical symbols

P Probability (P-value)

SE Standard Error

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1. INTRODUCTION

1. INTRODUCTION

Cull cow beef is a by-product of the beef and dairy cattle industries and includes animals that are slaughtered at > 30 mos. of age. These animals are culled for various reasons including poor health condition, poor temperament, failure to reproduce, and low productivity. Due to cull cow beef's inherent variation in carcass muscling, fat colour, fat thickness and skeletal maturity (ossification), their carcasses are usually downgraded to D quality grades in Canada (Mandell et al., 2006). As a result, cull cow beef is often used for ground beef production; this limits returns for beef cow-calf and dairy producers. Furthermore, the value of cull cows in Canada is still less than May, 2003 prices when BSE was discovered in Canada due to the past ban on the export to the U.S. of any beef from animals over 30 months of age including live animals. Many producers are still struggling to repay government loans while the numbers of cattle being exported to the U.S. have still not returned to pre-BSE levels (Broadway, 2008). Thus, there is a great need to increase utilization of cull cow beef domestically to improve producer returns as well as increase consumer demand. Demand refers specifically to the quantity of beef a consumer will purchase at a given beef price, with other factors held constant (Schroeder and Mark, 2000). However, due to the variability in tenderness, juiciness, and flavour of cull cow beef, grocery retailers do not market cull cow beef as steaks and roasts as a fresh product. According to the 1995 National Beef Quality Audit, the top five ranked beef quality concerns identified from surveys of beef purveyors, packers, restaurateurs, and retailers include 1) low overall uniformity and consistency; 2) inadequate tenderness; 3)

low overall palatability; 4) excessive external fat; and 5) high price for the value received (Smith et al., 1995). These concerns also carry over to cull cow beef quality; particularly with widespread tenderness, juiciness, and flavour inconsistencies. The lack of consistency in tenderness, and unsatisfactory eating experiences associated with cull cow beef are major problems for marketing whole muscle cuts from cull cow carcasses. The variability in the eating quality (tenderness, juiciness, flavour) of cull cow beef has long been recognized in the industry (Hodgson et al., 1992; Hilton et al., 1998), with maturity of the animal and body condition (leanness) being primary factors influencing quality (Shemeis et al., 1994). In terms of body condition score (BCS), a measure of weight/age and weight/height ratios with regards to proportions of muscle and fat, Apple (1999) suggested a BCS of at least 6.0 (scale 0-10) was needed to increase meat quality from cull cows and economic returns for cow calf producers.

The use of postmortem processing to improve quality, consistency, and eating experiences is necessary for the increase in returns and value of cull cow beef. Over the last 20 years, skeletal separation techniques were developed in which selected pre-rigor cutting of vertebrae, bone, fat, and connective tissue enabled muscles to be physically stretched by the weight of the hanging carcass. The stretching occurring with specific muscles results in increased sarcomere lengths and increases in beef tenderness. Stretching of the *longissimus* muscle increased sarcomere length and decreased shear force while increasing tenderness in youthful carcasses from steers and heifers (Wang et al., 1996; Ludwig et al., 1997; Aalhus et al., 2000). These select pre-rigor cuts may be incorporated into the standard kill floor procedures or in the chill cooler prior to the onset of rigor mortis.

The use of skeletal separation on cull cows was investigated by Mandell et al. (2006) with significant decreases in shear force for cooked *longissimus* muscle. Skeletal separation improved tenderness for the *semimembranosus* muscle but did not improve tenderness for other muscles from the round including the *semitendinosus*, *vastus lateralis*, *biceps femoris*, and *rectus femoris* muscles. Parallel studies had little success in using skeletal separation techniques to consistently decrease shear force for muscles from the round of youthful carcasses (Shank et al., 2002). Other studies found skeletal separation had little effect on reducing the shear force attributed to the connective tissue content of various muscles from the round (Claus et al., 1997; Shanks et al., 2002) nor have any impact on quantitative or qualitative collagen content. (Wang et al., 1994)

Postmortem ageing is well documented to improve beef quality including tenderness (Diles et al., 1994; Aalhus et al., 2004; Kolczak et al., 2008). In addition, Ludwig et al. (1997) postulated that skeletal separation procedures in combination with postmortem ageing may exert an additive effect for improving tenderness such that less ageing time may be required with skeletally separated carcasses to ensure tender beef. Further work conducted by Beaty et al., (1999) reported considerably lower WBSF values than those reported by Ludwig et al. (1997); however these authors suggested that using the skeletal separation techniques appeared to have no effect on the rate and extent of postmortem ageing. However, due to the simplicity of the carcass processing procedure, this technology has profound application possibilities for smaller scale beef packers and local meat shops to improve *longissimus* muscle tenderness (Beaty et al., 1999).

Injection processing or moisture enhancement of beef has been used to improve

product quality since the 1980's. Injection of calcium salts as well as commercial salt mixtures into beef has been used to improve beef tenderness, decrease Warner-Bratzler shear force and increase sensory evaluation ratings for *longissimus* muscle (Koochmaraie et al., 1990; Wheeler et al., 1992; Lawrence et al., 2003a). While use of calcium chloride was effective for improving beef tenderness, the calcium salt is not widely used by beef processors as a moisture enhancing solution since it deleteriously affects meat colour and meat flavour, as well as increasing meat purge (Wheeler et al., 1996; Lawrence et al., 2003a; Lawrence et al., 2004). Moisture enhancement via injection or dipping technologies have incorporated phosphate/lactate/salt solutions when processing fresh beef product. These treatments increase eating quality of beef via increased tenderness, juiciness and flavour of beef muscles from the chuck or round; or lower quality cuts including Canadian A grade or lower quality beef (Vote et al., 2000). The injection of phosphate/salt solutions successfully increased tenderness in *longissimus* and muscles from the round (Wheeler et al., 1991; Aktas and Kaya, 2001; Hoffman et al., 2008). Hoffman et al. (2008) found that infusion of a phosphate and lactate blend increased tenderness scores with steaks becoming significantly more juicy and flavourful than non-injected, control steaks. Although the injected steaks were rated more salty than controls, high consumer rankings for enhanced product have been interpreted to suggest that phosphates and lactates can be applied successfully to enhance the sensory attributes of young beef. Lamkey et al. (1993) postulated that merchandizing moisture enhanced beef prepared from muscles from the round and chuck could increase beef demand as consumers are willing to pay for moisture enhanced meat products that can improve tenderness and correct quality inconsistencies (Moeller and Courington, 1998; Robbins et

al., 2003). The injection of calcium salts including ascorbate and lactate was investigated by Lawrence et al. (2003a) for decreasing shear force via the activation of the calpain proteolytic enzyme system; suggesting that the cow-calf industry would benefit from using these postmortem processing methods that enhance the eating quality of cull cow beef to create a value-added product.

Previous research has investigated the use of skeletal separation and moisture enhancement on their own to enhance the eating quality of cull cow beef (Lawrence et al., 2003a; Mandell et al., 2006). However to our knowledge, no one has investigated combining skeletal separation and moisture enhancement techniques together to determine any additive effects on improving the eating quality of cull cow beef. The objectives of this study were:

To investigate the effects of skeletal separation and moisture enhancement procedures separately as well as together to determine any additive effects for improving the eating quality of cull cow beef including objective measures of meat quality and sensory evaluation of tenderness, juiciness, and flavour.

2.0 LITERATURE REVIEW

2.1.1 CONVERSION OF MUSCLE TO MEAT

After stunning and exsanguination, individual muscle cells are viable to maintain homeostasis and cellular respiration. In the living muscle cell, glucose is the primary source of energy while glycogen is the main storage glucose polymer. Glycogen is used by skeletal muscle for energy under anaerobic conditions (Hui et al., 2001). While the cell is alive, the production of two pyruvate molecules from glucose-1-phosphate is utilized in the Krebs cycle yielding reduced NADH molecules as well as ATP through oxidative phosphorylation taking place in the mitochondrial membrane of muscle cells. When oxygen is limiting, contracting living muscles in living muscle produce lactate which ordinarily would be exported to the interstitial space; the circulatory system can then transport lactate to the liver for gluconeogenesis or conversion to glucose-6-phosphate for energy usage. When a muscle cell is starved of oxygen-rich blood, its ability to import and export nutrients and waste products ceases, as what begins to occur during and after exsanguination. The cell cannot maintain homeostatic balance and is ultimately dying (Hui et al., 2001). Cellular characteristics including cytoplasmic pH changes, fluctuations in enzyme activity, loss of oxygen, and decreasing ATP synthesis will ultimately cause cellular death. A sudden influx of calcium ions from the sarcoplasmic reticulum will enter sarcomeres in the muscle and bind to proteins in the muscle cells causing rigor onset.

During exsanguination, the ability of muscle cells to receive oxygen via the blood supply (anaerobic conditions) and remove its newly formed lactate or lactic acid decreases over time. Building up within the interstitial fluid, lactic acid contributes to a drop in pH in animals before, during and after slaughter. The homeostatic balance that the muscle cells are trying to maintain becomes skewed and the hydrolysis of ATP, the breakdown of creatine phosphate, and the accumulation of lactate all contribute to a decline in antemortem and postmortem pH. The latter is responsible for the majority of the drop in pH (Shen et al., 2009). Ultimate post-mortem pH of muscle cells is dependent on the amount of muscle glycogen present within a muscle at slaughter and effectively represents the pH achieved after all energy stores are used while enzymes are still active. The initial pH of around 7.0 can drop to an ultimate pH of 5.5-5.7 in normal post-rigor beef depending on glycogen store amounts (Miller, 2007). Ultimate pH is very important for packers and consumers due to its impact on beef eating quality, water holding capacity, appearance, as well as microbial growth with implications on shelf life and food safety.

When ultimate pH remains high, above 5.87 in *longissimus* muscle, the meat is considered dark, firm, and dry (DFD) (Page et al., 2001). DFD beef can have a characteristic dark red, black, or sometimes purplish colour, and exhibits a dry and often sticky surface. The DFD beef is caused by lower than normal glycogen levels at slaughter where small amounts of lactic acid/lactate build up in muscle leading to a higher than normal, ultimate pH. When glycolysis occurs, two pyruvate molecules are produced along with NAD^+ . Anaerobically, lactic acid is produced as NADH is formed to re-enter the glycolytic cycle. Logically, the more glycolysis can occur, the more lactic

acid that will be formed under anaerobic conditions. The ability of a cell to break down glycogen according to how much glycogen is available is called the glycolytic potential. Glycolytic potential (GP), the indicator of metabolite activity within glycolysis was positively correlated to increased tenderness (Wulf et al., 2002). A high GP is indicative of an increased amount of glycolysis occurring with available glycogen estimated by the amount of lactic acid equivalence that is present. An increase in GP may help decrease the incidence of DFD in beef retail cuts. Palatability, in terms of juiciness for DFD was found to be similar in juiciness as that of normal beef but has less amounts of glycogen at the time of slaughter than that of normal beef (Wulf et al., 2002).

Water holding capacity is the ability of a tissue to hold its own water content during processing (Hui et al., 2001). Encompassing bound water, immobilized water, and “free” water, the bound water is attached to myofibrillar proteins via hydrogen bonds while immobilized water is held in place between myofibrils by capillary forces. Water designated as “free” water constitutes less than 20% of the total water, “free” water is unimpeded and can flow through the muscle tissue freely. DFD beef’s lean surfaces and dry appearance throughout hold much more water than what is found in beef muscle with a normal ultimate pH (5.4-5.7). Although DFD meat is dark, firm and dry, it is a paradox because the dry texture does not accurately represent the meat’s high water-holding capacity. The extent of postmortem pH decline directly affects water holding capacity; the higher the ultimate pH, the smaller decrease in water holding capacity. Rate of pH decline also contributes to the determination of water holding capacity as denaturation of sarcoplasmic proteins increases as rate of pH decline is increased. The fast rate of ATP breakdown (i.e. pH decline) increases the tendency for actomyosin to contract (Penny,

1977) as it forms and will cause expulsion of extracellular fluid laden with dissociated proteins (Bendall, 1960). The use of all the glycogen stores and the loss of ATP postmortem (onset of rigor mortis) can facilitate formation of actomyosin as muscles enter rigor mortis causing loss of water holding capacity. This is due to actomyosin having a lower water holding capacity than actin or myosin and because lower ATP levels initiate denaturation in those proteins where structural integrity is dependent on ATP energy (Lawrie & Ledward, 2006). The rate at which postmortem pH drops can also have a direct effect on the water holding capacity of meat. Along with increased denaturation, a drop in pH (increase of ATP consumption) enhances the tendency of actomyosin to contract and expel dissociated proteins to the extracellular fluid.

In all meat products, water holding capacity affects the appearance of meat before and after cooking, its behaviour during cooking and the juiciness felt during mastication. Most of the moisture within muscle tissue resides in the spaces between the thick filaments comprised of myosin and the thin filaments of actin and tropomyosin (Offer et al., 1984; Hui et al., 2001; Lawrie & Ledward, 2006)). This water is held in place by capillary forces while no more than 5% of the total water within muscle may be directly bound to the existing hydrophilic groups on the amino acids of the proteins (Lawrie & Ledward, 2006). Water can exist as “bound” or “free” water within the muscle. During the onset of rigor mortis, the proportion of bound water is not changed significantly while the free water from the intracellular space is moved to the extracellular areas. Interfilament spacing greatly affects the amount of water holding capacity for each myofibril. The interfilament spacing in a myofibril is mainly determined by long range electrostatic forces; forces of charged ions or proteins that hold a specific conformation

or position within the spacing. Some of the forces are against swelling of the interfilament spacing whenever negative charges on the protein filaments exist at an elevated pH. These include attachments of the actin filaments to the z-line, myosin filaments attaching to the M-line proteins, and cross-linkages being formed between the actin and myosin filaments (Lawrie & Ledward, 2006). Since all proteins contain amino acids with positive or negative charges, the overall protein charge is directly related to the pH. Generally, a lower pH value yields proteins with a net positive charge while a higher pH value suggests a net negative protein charge. When muscle proteins have a net neutral charge (termed the isoelectric point [pI]), the ability of these proteins to bind free water is minimal when the pH equals that of the pI. The isoelectric point equals the pH when pH is about 5.4 in proteins (Faustman and Swartz, 2007). When pH = pI, meat will have a minimal water holding capacity; the product will tend to be more dry and weigh less, potentially lowering economic value.

DFD beef's high ultimate pH is directly linked to the respiration of mitochondria which consume oxygen and form deoxymyoglobin. The deficiency of glycogen within postmortem muscle results in a higher pH (> 5.87) and an overall darker appearance which alters the light absorption on the muscle's lean surface producing a darker, sometimes dark red colour we attribute to dark cutters (Ashmore et al., 1971). Normal beef muscles that appear to have more moisture content actually have less moisture than DFD beef (Du et al., 2009). Due to the drier and often stickier surface of the lean muscle in DFD beef, the lower amount of denaturation of myoglobin can facilitate increased aerobic bacterial metabolism at the surface, decreasing shelf-life of a packaged meat product. Water holding capacity is also known as weep, drip loss, or purge and can vary

depending on muscle type, the treatment the muscle has received and (or) age of the animal when slaughtered. Depending on the age of the animal prior to slaughter, the *longissimus* muscle produced more weep in younger cattle (18 mo) vs. older cattle (54 mo) (Powell, 1991). The authors suggested that to minimize weep in chilled, vacuum packed primals, meat processors should use the older and heavier age group of cattle whilst not freezing the meat during storage or distribution. Muscle fibers from mature cattle have an increased amount of cross links between the actin and myosin heads. The tighter interfilament spaces may limit the amount of moisture that can be retained postmortem. Cooking of meat results in loss of moisture; this loss of moisture, termed cook loss, represents the amount of moisture lost from the initiation of the heating process until the meat has reached its designated endpoint temperature. The typical endpoint temperature for a medium rare piece of meat used for shear force evaluation is approximately 69 -70°C (Wheeler et al., 1997a). Cook loss percentages are inherently affected by the degree of doneness and will vary according to the product endpoint temperature. Parrish et al. (1973) studied the effects of internal temperature for doneness on beef *longissimus* steaks and found that an increase in endpoint temperature corresponded to an increase in cook loss.

Cattle that are rested antemortem and not subjected to high levels of stress have a minimum of 100 ul/g of glycogen in muscle tissue; this is the threshold value for glycogen potential before the muscle is considered a dark cutting muscle (Wulf et al., 2002). Glycogen stores can be used up or be drastically depleted before slaughter by cattle subjected to a variety of pre-slaughter stressors. This can result in the production of DFD beef as there is limited substrate for postmortem lactic acid production. Stressors

which can contribute to production of DFD beef include mixing of unfamiliar cattle, loading, transport, lairage and lairage time, fasting, antemortem stunning to render the animal unconscious, chilling, as well as electrical stimulation immediately following carcass cooling (Du et al., 2009). Controlling these variables can help reduce the incidence of DFD beef in any packing plant. According to recent National Beef Quality Audits, the incidence of dark cutters in the U.S. ranged from 1.9% (Garcia et al., 2008) to 2.3% (McKenna et al., 2002), with dark cutters totalling 697,130 head. The incidence of dark cutters is decreasing possibly due to research studies which have identified factors involved in influencing glycogen levels (Bartos et. al., 1993; Wulf et al., 2002; Apple et al., 2005).

Predictable antemortem and initial 24 h postmortem pH levels are essential for producing high quality meat. An ultimate pH of 5.3-5.7 represents the vast majority of quality beef that is associated with desirable lean colour. In a survey incorporating data from 3 packing plants in Illinois, Texas, and Ohio, objective measures of color, L^* a^* b^* were negatively correlated to muscle pH in 1062 steers and heifers (Page et al., 2001) with lower L^* values as pH decreased. Purchas (1990) examined the role of pH for determining relative tenderness of *longissimus* muscles in bulls and steers and implicated that water holding capacity was also influenced by cooking temperature and ultimate pH. There was a curvilinear relationship at medium cooking temperature (65°C) with increases in tenderness, and a linear increase in water holding capacity at higher temperatures (90°C) (Purchas, 1990). Ultimate pH may affect tenderness via effects on proteolytic activity, since calcium activated proteases have an optimum pH closest to 7.0 (Purchas and Aungsupakorn, 1993). Increases in ultimate pH values may be associated

with an increasingly favourable environment for the proteases to operate. Animal management and post-mortem processing need to be regulated to control rate and extent of post-mortem pH decline and their impact on beef colour and tenderness to prevent deleterious effects on visual acceptability and(or) eating quality.

2.1.2 SARCOMERE LENGTH, COLLAGEN, AND TENDERNESS

The skeletal muscle cell is a mass collection of myofibrils organized in a repeating fashion. Each myofibril is a string-like structure consisting of numerous repeating units called sarcomeres. The sarcomere is made of several types of proteins which interact to form an A-band and two I-bands. The length of the sarcomere otherwise known as sarcomere length is measured as the distance between two longitudinally adjacent Z-lines (Du et al., 2009). Sarcomere length has been documented to affect myofibrillar tenderness in *longissimus* muscle (Rodbotten and Hildrum, 2000), with lower sarcomere lengths associated with lower tenderness. However, another study showed longer measurements of sarcomere length do not always result in tender meat (Soerheim and Hildrum, 2002). Sarcomere length can shorten by temperature decreases during carcass chilling. Marsh and Leet (1966) reported decreases in sarcomere length with pre-rigor excised, 'red' muscles or muscles with higher amounts of myoglobin and mitochondria (Lawrie and Ledward, 2006). For beef and lamb, appropriate chilling parameters for air temperature and velocity are needed to minimize cold shortening; this can be accomplished by ensuring muscle temperatures do not fall below 10°C before the pH reaches 6.2 (Ali et al., 2008).

In skeletal muscles, myofibres (muscle fibres or cells) are bound together in a cluster of bundles; each myofibre is surrounded by a fine layer of connective tissue called the endomysium. The myofibres are then grouped together in fascicles by a tough layer of connective tissue known as the perimysium. Furthermore, the perimysium and fascicles are covered by another layer of connective tissue called the epimysium. All layers contain collagen, elastin, and fibroblasts. The connective tissue is comprised primarily of collagen which is believed to contribute to the so-called “background” toughness of meat (Kruggel et al., 1970; Torrescano et al., 2003). Texture can be described as the function of the size of bundled fibers into which perimysial septa of connective tissue divide the muscle longitudinally (Hammond, 1932). In general, coarse grained muscles that have rapid post-natal growth, like the *semimembranosus*, have larger muscle fiber bundles than slower growing *semitendinosus* muscle fibers of smaller size (Lawrie and Ledward, 2006). Rapid growth rates may influence muscle tenderness by reducing connective tissue’s contribution to background toughness. The amount of perimysium around a muscle fiber bundle can also determine relative coarseness as a thicker perimysium layer represents a coarse muscle fiber appearance (Ramsbottom and Strandine, 1948). Assuming cold shortening is controlled or avoided, the general hypothesis includes that collagen is also a major determinant of the texture and mouth-feel of meat and that variations in texture can be dependent on the amount of collagen present within a muscle. Total collagen and insoluble collagen contents for *semitendinosus* (ST), *semimembranosus* (SM), *longissimus et lumborum* (LTL), and *psoas major* (PM), were both positively correlated with Warner-Bratzler shear force values (Torrescano et al., 2003). The ST contained more total collagen than PM with

higher WBSF values; however, soluble collagen contents increased markedly for both with postmortem ageing (Kolczak et al., 2008). Sensory attributes including texture were also determined for the ST and PM with chewiness and hardness decreasing with increased postmortem ageing (Kolczak et al., 2008).

Shear force measurements and eating quality in terms of tenderness, juiciness and flavour are often profiled and studied together because of the attributes that accompany shear force in explaining tenderness variability. As a benchmarking standard, Warner-Bratzler Shear force is used to gauge the amount of force that is required to cut perpendicularly through the muscle fibers such as to mimic the compression created when biting down on a piece of meat during the mastication process. Warner-Bratzler Shear force (WBSF) is often used as a universally standardized method for assessing tenderness in meat samples. The process of determining WBSF includes cooking steaks to a common endpoint temperature and then chilling the cooked product overnight. Cores of a standard diameter are prepared with a minimum of 6 cores per steak sheared using a Warner-Bratzler blade at a head speed of 200 mm/min (AMSA, 2005). The amount of force required to completely shear a sample core is measured as the amount of mass required to shear it in half on a designated WBSF machine. Shear force is commonly recorded in Kilograms (Kg) or Newtons (N). Tenderness of steaks can then be estimated and ranked according to the amount of force needed to shear them. Wheeler et al. (1997a) suggested that the implementation of a standardized WBSF protocol will not only increase the comparison of multiple institutions' data but also increase repeatability of obtained results. Oury et al. (2009) used the physicochemical characteristics of the *rectus abdominis* muscles to correlate WBSF, tenderness, juiciness,

flavour and shear force in Charolais heifers. Tenderness was inversely proportional to total collagen and lipid content, while juiciness was positively correlated with lipid content. Flavour and shear force values were correlated with total collagen, lipid and 27K proteasome contents (Oury et al., 2009). The researchers reported that 33% of the variation in tenderness could be explained by the total collagen, lipid, and proteasome contents included in a multiple regression model with both tenderness and lipid content increasing as total collagen content decreased. Jurie al. (2007) used sensory evaluation to determine muscle differences in total collagen, overall tenderness, and juiciness for steaks from Holstein and Saler cull cows. Total collagen content was highest in the *semitendinosus* followed by the *semimembranosus* and *triceps brachii*. The panellists rated the *longissimus* thoracis for highest initial overall tenderness while the *semitendinosus* had the least. In general, the panellists found the highest ranking for tenderness associated with samples containing lower amounts of total and insoluble collagen (Jurie et al., 2007). Muscle type accounted for the greatest variation in meat quality from dairy and beef cull cows when slaughtered at the same age and fat score.

2.1.3 POSTMORTEM AGING EFFECTS ON MEAT TENDERNESS

Three major factors that affect meat tenderness include “background tenderness”, the toughening phase, and tenderization phase (Du et al., 2009). Although background toughness from many factors such as collagen content and muscle type is present at the time of slaughter, the toughening and tenderization phases occur during the process of postmortem ageing. Postmortem ageing by definition is the practice of holding meat at

low temperatures in coolers for various lengths of time to improve tenderness. This has been practiced for over a century but is still considered by most to be variable by species and even by breed (Novakofski and Brewer, 2006). Tenderness is positively correlated with postmortem ageing time across breeds. However, breed differences in proteolytic enzymes will result in breed differences in connective tissue breakdown and background toughness. The “tenderization” that occurs during the aging process is mainly due to the hydrolysis of myofibrillar proteins by the calpain enzyme system found in the animal (McCormick, 2009). The tenderization process begins at the time of slaughter and continues until the end of ageing. The calpain enzyme system includes calpain enzymes responsible for endogenous protein breakdown and calpastatin, the inhibitor of the calpains. The calpain enzymes are calcium-dependent proteinases involved with structural weakening of muscle proteins and protein turnover during the aging process. Consisting of two enzyme isoforms, micro-calpain and milli-calpain, the calpain enzyme system requires different concentrations of calcium ion concentrations for activation (McGregor, 2008). Micro-calpain requires as little as 1-20 μM while milli-calpain requires 250-750 μM of calcium ions present for binding and activation to occur (Goll et al., 2003). Evidence has been shown to link micro-calpain activity as a mechanism of postmortem tenderization and degradation of myofibrillar proteins (Geesink et al., 2006). Calpastatin is a specific endogenous inhibitor of micro-calpain and milli-calpain that requires existing calpains to bind to calcium for its activation within skeletal muscle. The regulation of skeletal muscle turnover is regulated through the interaction of calpastatin with calcium bound calpains. Calcium ions have a dual role within postmortem muscle; Ca ions induce rigor-contraction by the increase of sarcoplasmic Ca ion concentrations to

300-500 μM . In addition, increases in Ca ion concentrations up to 100 μM cause weakening of myofibril structures, desmin intermediate filaments and potentially the endomysium, increasing meat tenderness (Takahashi, 1996). These functions of Ca further increase tenderness through their ability to restore sarcomere shortening, indicating weakening of rigor linkages between actin and myosin (Takahashi et al., 1995). Breed differences in calpain and calpastatin enzyme activities are present which are partially responsible for breed differences in tenderness. Calpastatin, the inhibitor of the calpain enzymes which are activated by Ca^{2+} , and its activity is genetically determined in some breeds of cattle with a calpastatin gene correlating with toughness (Bishop et al., 1993; Shackelford et al., 1994). Breeds that express the calpastatin gene to a greater extent have less calpain enzyme activity in their muscles. *Bos indicus* cattle have greater amounts of calpastatin in muscle than *Bos Taurus* breeds with increased inhibition of calpain enzymatic action leading to tougher meat (Shackelford et al., 1991).

Postmortem aging increases degradation of the collagen network (Nishimura et al., 1995), decreasing the breaking strength of the perimysial connective tissue in raw meat (Lewis et al., 1991). Lewis et al., (1991) showed that the temperature at which meat is cooked at can alter the connective tissue's resistance to shear force. During postmortem aging, proteoglycans are also degraded, resulting in the weakening of perimysium and the separation of collagen fibers from endomysium and perimysium (Nishimura, 1996). Structural weakening of the myofibrils, the intermediate filaments and intramuscular connective tissues such as the endomysium and perimysium have been shown to contribute to the tenderization process during postmortem ageing (Takahashi K, 1996). The amount of connective tissue in muscle, sarcomere length, and proteolysis of

myofibrillar proteins account for the majority of the variation observed in tenderness of aged meat. Furthermore, the contribution of each of the factors to the tenderization process is thought to be variable. Tenderization of the *psoas major* is believed to be determined by sarcomere length while tenderization of the *longissimus* muscle is predominantly determined by the degree of proteolysis during ageing. Connective tissue content is a major contributor to the tenderness of the *semimembranosus* (Koohmaraie et al., 2002). Although ageing is accepted as a means to increase beef tenderness, individual muscles may not necessarily benefit greatly from set ageing periods. Aalhus et al. (2004) studied postmortem ageing up to 21 days in bovine *longissimus thoracis* and *semimembranosus* muscles. While tenderization of both muscles benefited from the process of ageing, shear force did not decrease for the *semimembranosus* as muscle aging increased. Excessive moisture loss in the *semimembranosus* may have played a role in the difference in tenderness increases throughout the 21 day period (Aalhus et al., 2004).

2.1.4 CULL COWS AND GRADING

Cull cows are removed from the herd (culled) for different reasons including age, poor performance, bad temperament, poor health or condition, and reproductive failure (Stelzleni et al., 2007). Cull cows account for as much as 15 to 20% of total revenues for all beef production (Sawyer et al., 2004) with 4.86 million head of cull beef and dairy cows entering the slaughter market from the U.S domestic beef supply in 2005 (USDA, 2005). In 2009, over 0.3 million head were sent to slaughter at below market prices (Stats Canada, 2009). In Canada, cull cows of D1-D4 grading can have up to 15 mm of

subcutaneous fat and a white to yellow appearance. Cull cows are often mature (over 30 mos.) and are of grade D1 or lower in quality when graded through the Canadian Beef Grading Agency. In Canada, Grade D1 or lower beef products are not graded in terms of their ribeye and subcutaneous fat colour content as what is seen for higher graded beef product. In contrast, youthful carcasses rated as Prime, AAA, AA, A, or B grading are subject to more stringent requirements for ribeye colour, good to excellent muscling which affects meat yield, marbling ranging from traces to slightly abundant, and being from cattle carcasses no older than 30 months of age. Grading is intended to place carcasses into uniform groups of similar quality, yield, and value, in order to facilitate marketing and production decisions (CBGA, 2010).

In terms of cull cow eating quality, tenderness, juiciness and flavour vary greatly according to cull cow age. A study involving 15-24 month old bulls and 4-8 year old french cull cows was conducted evaluating *longissimus*, *semitendinosus* and *triceps brachii* muscles for meat quality attributes; tenderness rankings were lowest for cull cows and highest in the youngest bull age group (Dransfield et al., 2003). Juiciness and flavour rankings were higher in the oldest age group of cull cows and bulls versus younger age groups. Beef muscles from older cull cows tend to be tougher and drier upon first bite and may impart a mealiness residue in comparison to more youthful animals (Hoffman, 2006). Variations in tenderness when comparing cull cows to youthful beef carcasses is partially attributed to the inherent strengthening of the collagen cross-linkages that occurs as animals age (McCormick, 1994). Variation in tenderness also exists amongst cull cow carcasses; this has been evaluated for muscles, from the *longissimus* and round (Stolowski et al., 2006). These authors found muscle type was a

major determinant for overall tenderness. Steaks from the round had a higher WBSF than *longissimus* muscle. Extensive variation in eating quality (tenderness, juiciness, and flavour) for cull cow beef only exacerbate the issue of trying to increase value of cull cow beef in addition to the already degraded rating given to mature beef by the Canadian Beef Grading Agency.

2.1.5 LIPID OXIDATION AND COLOUR

Lipid oxidation can be defined as a process initiated at the membrane level in phospholipid fractions as a free-radical autocatalytic chain mechanism where pro-oxidants interact with unsaturated fatty acids resulting in the generation of free radicals and propagation of the oxidative chain (Campo et al., 2006). Lipid oxidation impacts meat quality as it is a major contributor to flavor and colour deterioration in meat, and limits product storage and shelf life in the presence of oxygen when meat is refrigerated or frozen. Campo et al. (2006) examined the relationship of lipid oxidation to positive attributes of meat (beef flavour, overall palatability); display time in the retail meat case was negatively correlated to palatability attributes by a trained sensory panel. Lipid oxidation in meat occurs primarily by auto-oxidation where the rate will vary depending on different surface and internal conditions of the meat. Auto-oxidation is the major oxidation mechanism in meat and begins occurring at the membrane level within phospholipid fractions (Campo et al., 2006). Lipid oxidation is initiated by reactive oxygen species such as hydroxyl radicals and can form lipid radicals by removing a hydrogen atom from the fatty acyl group of polyunsaturated fatty acids (Du et al., 2009).

Since these lipid radicals have a strong enough reactivity from the initial reactions, free radicals can be transferred to adjacent lipids perpetuating a cascade of transfers. To prevent these transfers, the free radicals must be quenched to effectively stop the reactions. This can be accomplished by use of antioxidants (Gatellier et al., 2001; Harris et al., 2001) by supplying vitamin E into finishing beef diets to minimize lipid oxidation and increase colour stability. Malondialdehyde (MDA) is a secondary oxidation product of polyunsaturated fatty acids; MDA is one of the lipid oxidation products that are known to be toxic and carcinogenic. Lipid oxidation products can also have a negative impact on meat palatability and flavor. The importance for prevention of lipid oxidation within the meat industry should not be discounted.

There are various methods for measuring lipid oxidation of fatty acids including distillation methods, acid extraction methods, and HPLC methods to determine amounts of primary and secondary by-products of lipid oxidation (Witte et al., 1970; Squires et al., 1991; Raharjo et al., 1993; Botsoglou et al., 1994). The thiobarbituric acid (TBA) test is the most common method for measuring MDA equivalents from lipid oxidation in food products and biological samples according to Botsoglou et al. (1994). This quantitative test is based upon spectrophotometric determination of a pink MDA fluorescent product complex formed after the reaction of MDA with two molecules of TBA. Substances called thiobarbituric acid reactive substances (TBARS) are indicator components that react with TBA to produce this pink complex. The test can be performed in multiple ways including: simple heating of TBA, distillation of sample followed by reaction of distillate with TBA, or by extraction of the lipid portion of the sample with organic solvent and the extract reacted with TBA. All methods are rapid and efficient for

estimating the extent of lipid peroxidation in various food and biological samples (Botsoglou et al., 1994). The wide availability of modern spectrophotometers allows for easy generation of MDA-derivative spectra and provides a reliable way to determine the amount of lipid peroxidation that is occurring. The TBARS method is an effective way to measure lipid oxidation for meat based on its simple procedure and high sensitivity. A standard TBARS assay which yields less than 2 MDA equivalents per Kg meat sample may be used as an indicator threshold for acceptable oxidation in beef. However, Cheng and Ockerman (2007) found that the presence of 0.10 mg of MDA/Kg in a beef roast was associated with evidence of cardboard or off-flavour. Warmed-over flavor refers to the quality deterioration of cooked, pre-cooked or frozen meat products and often includes development of undesirable flavours (the warmed-over flavour) concurrent with loss of desirable meat flavour characteristics (Spanier et al., 1988; St. Angelo et al., 1990). Warmed-over flavour can be detected as cardboard-like, painty, rancid or stale. Cheng and Ockerman (2007) examined use of additives to limit warmed-over flavour and recommended a combination of vitamin E, ascorbic acid and phosphate at 0.3% to reduce lipid oxidation and to prevent warmed-over flavours.

The colour of meat can be measured using various subjective, objective, and (or) instrumental methods to grade and analyze colour variations in meat samples. Colourimeters and spectrophotometers can be used to measure the colour of meat, utilizing numerous measurement standards such as HUNTER, CIE, or tristimulus values, various illuminant observers and aperture sizes (Brewer et al., 2002; Mancini and Hunt, 2005). Meat researchers often measure colour using Hunter or CIE L^* a^* b^* system with L^* or Lightness, a^* or the continuum from red to green (with red being desired) and b^* or

the continuum from yellow to blue (with yellow being desired). Values for the latter two traits are used to calculate Hue ($^{\circ}$) angle and chroma (c^*) values. Hue angle describes the general type of colour based on the wavelength of light radiation and represents the degree to which a stimulus can be associated to L^* , a^* , and b^* values. Chroma (c^*) values represent overall color purity. These measurements can be easily referenced to a standardized calibration plate and used to accurately measure each component on a uniform scale. Colour measurements are used in meat research to develop processes to stabilize oxidization as well as increase overall shelf life.

Meat colour is considered one of the most important factors when purchasing meat at the consumer level and has a major impact on retail sales and consumer satisfaction (Lynch et al., 1999; Gatellier et al., 2001). A major goal for retail outlets is to extend display time in the meat case to ensure the cut/package is sold. Colour stability in meat can be a deciding purchasing factor at the consumer level as consumers use colour to indicate freshness and palatability. To an average consumer, a bright red cherry colour is considered as the standard for fresh beef (Lynch et al., 1999). Since pigmentation in beef is principally due to the oxidation state of myoglobin, it is in the retailer's best interest to delay oxidation of oxymyoglobin to metmyoglobin. Conversion of oxymyoglobin to metmyoglobin changes meat surfaces from cherry red to metmyoglobin's distinctive "un-fresh" appearance of light to dark brown. Lynch et al. (1999) found metmyoglobin formation positively correlated with lipid oxidation; the process appears to be dependent on antioxidant status of myoglobin in a meat sample. To prevent formation of metmyoglobin, researchers have examined methods to inhibit or minimize lipid oxidation, maximize colour stability, and maximize shelf life including

α -tocopherol (vitamin E) supplementation in pre-slaughter diets, moisture enhancing (pumping) beef with food additives such as calcium chloride, or use of modified atmosphere packaging (MAP) which provides a controlled atmosphere to prevent product spoilage from occurring through oxidation (Gatellier et al., 2001; Eichenberger et al., 2004; Lund et al., 2007). Gatellier et al. (2001) investigated α -tocopherol acetate (Vitamin E) supplementation in 32 to 44 mo old Charolais cattle for 111 d prior to slaughter. *Longissimus* muscles became discoloured in beef from cattle not supplemented with vitamin E or beef not packaged using MAP. When α -tocopherol acetate supplementation was included with MAP, discolouration was decreased in the *longissimus thoracis* and *triceps brachii*. Furthermore, TBARS values were significantly lowered at the end of the storage times when cattle were supplemented with α -tocopherol acetate. Lund et al. (2007) investigated using rosemary or 1:1 ascorbate/citrate in combination with MAP (100% N₂, 80% O₂/20% N₂) to reduce lipid oxidation in minced beef patties during dark storage for 6 d. The ascorbate/citrate treatment in combination with MAP protected the patties from lipid oxidation but not from protein oxidation. In the presence of high oxygen concentrations, use of ascorbate/citrate protected fresh red meat colour better than using rosemary. The use of ascorbate (vitamin C) or vitamin E solution mixed or possibly injected into beef may help to reduce lipid oxidation and potential discolouration to increase shelf life.

To exacerbate the problem with meat colour stability within the retail market, there is also a problem with colour stability in retail cuts from mature (> 30 mo) cattle. Xiong et al. (2007) noted the importance of beef harvested from cull beef and dairy cows for the meat industry around the world. Mature cow beef is used extensively for ground

beef production and processed beef products; cow beef may be utilized for various retail cuts if the problems with tenderness, oxidation and colour stability can be addressed. The *longissimus* muscle tends to be darker in colour when sourced from older animals vs. younger cattle. Xiong et al. (2007) examined lipid oxidation and tenderness in beef from cattle aged 2-12 yr. Lean colour, oxidative stability and tenderness in the *longissimus*, *semitendinosus* (ST) and *semimembranosus* (SM) muscles decreased as cattle increased in age. It was successively found that muscle location on a carcass and its particular muscle type can affect similar attributes for all cattle ages. (Xiong et al., 2007; Schreurs et al., 2008). The ability to control lipid oxidation and decrease colour deterioration should assist the retail market by increasing shelf life for beef sourced from all ages of cattle and increase the value of mature beef retail cuts.

2.1.6 MICROBIAL SAFETY STUDIES

Micro-organisms exist within and on all animals that are used for meat based products. Sources of most pathogens include those from the gut of an animal, the hide, and other external body parts that come in contact with a contaminated surface; post-processing this could include any meat surface that is exposed to the air. During slaughter, the removal of the gut and hide is done to minimize cross contamination with the carcass being dressed. The presence of large numbers of micro-organisms on the hide makes the hide pulling procedure a critical control point in terms of food safety. The contamination of *Listeria monocytogenes* on food processing equipment and *Escherichia coli* through indirect fecal matter contact is avoided through the use of sanitary protocols

implemented by meat processing facilities. When the meat products are handled and processed into smaller retail cuts, the possibility of contamination lies with the processor. Procedures including washing hands, trimming meat product, utilizing decontamination of tools as well as sanitizing working surfaces with chlorine or organic acid treatment helps minimize the contamination that may occur (Fung et al., 2001).

Meat products themselves are environments suitable for specific types of microbial growth. The exposed lean tissue from a retail cut provides readily available sources of energy, carbon and nutrients. The pH value, typically in the range of 5.5-6.5 is ideal for growth of most bacteria, however, growth rate may be slightly reduced at lower or higher (DFD beef) pH (Varnam and Sutherland, 1995). Although this exerts a range effect, it is generally considered to have little effect on gross composition of microflora. In practice, extrinsic factors including refrigeration, vacuum and modified atmosphere packaging is done to limit the bacterial growth of present microflora after processing is carried out. In air, the shelf life of meat stored at a refrigerated temperature ($<5^{\circ}\text{C}$) is limited by microbial growth of gram-negative, psychrotrophic, and aerobic rod-shaped bacteria with off-odours generally appearing when growth numbers reach $10^7/\text{cm}^2$ while slime is visible at $10^8/\text{cm}^2$ (Varnam and Sutherland, 1995).

Research conducted studying the reduction of micro-flora including *E. coli* and various other aerobes was completed (Anderson and Marshall, 1990) to determine the usefulness of lactic acid and temperature on carcasses when they are being dressed. Lactic acid at 3% and 70°C was most effective in reducing aerobic bacteria while *E. coli* was affected, but to a lesser extent. Contrarily, research done by Gill et al. (2003) determined the decontamination ability of 2% lactic acid, vacuum-hot water cleaning and

trimming and found them ineffective. Furthermore, washing reduced high levels of bacteria but was ineffective in removing an existing low amount of bacteria. They suggested that the most efficient and effective way to reduce bacteria on carcasses is by washing and pasteurizing with steam or hot water without other treatments. A later study by Gill et al. (2008) showed that injection of brine solution with *Listeria innocua* bacteria without mechanical tenderization yielded a decrease in bacterial numbers with decreased brine pressure and an increase in injector strokes.

While meat is in retail packaging, the retail shelf life is limited by the spoilage due to microbial growth occurring over time. Extension of shelf life by the control of aerobic bacteria has been seen with cooked top rounds injected with 1-4% sodium lactate solutions allowing shelf-life up to 84 days (Papadopoulos et al., 1991a). Follow up trained sensory evaluation panels found that the treated rounds were more beefy and flavourful than that of rounds not injected with sodium lactate (Papadopoulos et al., 1991b). Another method tested to control aerobic bacterial growth on meat samples in the retail display package was completed using lactic acid bacteria to determine the effects of their presence on *Listeria monocytogenes*. The addition of lactic acid decreased *Listeria monocytogenes* bacterial counts by 5 log CFUs (Djenane et al., 2005). The use of lactic acid as a bacteria or a surface treatment solution may help decrease overall bacterial counts on carcasses being dressed or retail cuts being displayed in their respective packaging.

2.1.7 CARCASS SUSPENSION/SKELETAL SEPARATION

Beef carcasses are traditionally suspended by the Achilles tendon in commercial packing plants worldwide. Problems with tenderness inconsistencies for *longissimus* muscle and muscles from the round for youthful cattle (steers and heifers) stimulated research examining various methods of cutting vertebrae, connective tissue, and muscle tissue to essentially stretch targeted muscles for tenderness enhancement. When the carcass is treated with cuts to ligaments, bones, and surrounding fat, the suspended muscle bears the weight of the forequarter, generating a greater amount of stretch on the isolated muscle versus an intact carcass side. Various methods of carcass suspension have been investigated for their effects on meat quality and muscle fiber properties including suspending the carcass from the aitch bone (obturator foramen) (Hostetler et al., 1970a; Hostetler et al., 1970b; Hostetler et al., 1972; Hostetler et al., 1973; Sørheim et al., 2001). The Texas A & M Tenderstretch™ method developed by Hostetler et al. (1970) had a profound effect for increasing sarcomere length and sensory-panel tenderness and softness scores (Hostetler et al., 1972). By suspending the carcass from the aitch bone, the weight of the carcass was effective in increasing sarcomere length, decreasing Warner-Bratzler shear force values, and producing more tender sub-primal cuts for retail sale. Nevertheless, this method of suspension for improving tenderness by stretching the carcass requires abattoir modifications and may alter appearance of key retail cuts (Aalhus et al., 1999).

For the past two decades, research has included experimental implementation of carcass stretching, with selected cuts on the carcass being made to cause stretching of

muscles; this process has been referred to as skeletal separation. Dransfield et al. (1991) examined 3 postmortem processing techniques with rapid chilling including electrical stimulation, hip suspension and ageing for their effects on pork quality. Rapid chilling without electrical stimulation increased toughness for *longissimus* in the lumbar region but not in the *semimembranosus*. Electrical stimulation in combination with suspension from an “S” shaped hook through the obturator foramen (pelvic suspension) improved tenderness in both muscles (Dransfield et al., 1991). Eikelenbloom et al., (1998) evaluated pelvic suspension and cooking temperature on tenderness of electrically stimulated beef. Compared to steaks from control carcass sides, they found that shear force values increased for steaks sourced from pelvic suspended carcass sides. The authors suggested that in electrically stimulated and aged beef, pelvic suspension had opposing effects on myofibrillar and connective tissue contributions to shear force value. Collagen strength increased and myofibrillar strength decreased from the treatments which were likely due to the spatial organization of the collagen fibers.

Claus et al. (1997) investigated the stretching effects of pre-rigor manipulation of *longissimus* muscle using the Tendercut™ process. This process requires cutting all tissues surrounding the LM at the 12th Thoracic vertebra, allowing the LM itself to bear the weight of the forequarter. The 12th vertebra is severed identically to carcass processing before USDA and CBGA quality grading (Claus et al., 1997), except this procedure is performed soon after the carcass is dressed rather than after a period of carcass chilling. The treatment may incorporate another skeletal cut through the ischium on the pelvic bone at the round/loin muscle junctions. These two cuts are implemented due to the ease at which this treatment could be conducted to enable a sizeable stretch to

the selected muscles before the onset of rigor, and without modification of existing infrastructure for carcass suspension in commercial packing plants (Claus et al., 1997) . The Tendercut process decreased within muscle variation in tenderness for the *Longissimus* et thoracis (LT) and the *Longissimus* et lumborum (LTL) as well as increased overall tenderness for the *rectus femoris* (RF) and the *gluteus medius* (GM) (Claus et al., 1997). Skeletal separation using the Tendercut process did not affect yield grade, CIE L*a*b* values, chemical states of myoglobin, purge and cooking losses, and total collagen while improving tenderness along the entire length of the *longissimus* muscle in steers (Ludwig et al., 1997). Beaty et al. (1999) used the Tendercut procedure on Brangus heifers and found a 33-38% decrease in Warner-Bratzler shear force values for ribeyes and strip loins versus untreated, control sides. Sarcomere length values increased 35.8 and 33.2% respectively for rib eyes and strip loins.

A modified Tendercut procedure was used for skeletal separation of the round to evaluate process effects on the *vastus medialis*, *vastus lateralis* and the *rectus femoris* (Wang et al., 1994). Sarcomere lengths increased and shear force decreased for all 3 muscles with the Tendercut procedure, while there was no effect of skeletal separation on collagen solubility, cooking losses, or thawing losses (Wang et al., 1994). Sarcomere lengths for muscles from the round including the *semitendinosus* and *semimembranosus* increased as much as 5-7% in the Tendercut treated carcass sides (Beaty et al., 1999). However, there was no effect on shear force compared to control carcass sides for these muscles from the round. The Tendercut process has potential to be included into future abattoir operations to increase sarcomere length and improve beef tenderness for the *longissimus* muscle when applied within 2 hours following slaughter during rapid chilling

(Beaty et al., 1999).

Aalhus et al. (1999) pointed out a major limitation of the Tendercut process in that the location of the prerigor cut at the 12th vertebra will interfere with the Canadian grading site, possibly affecting the grading process and altering the overall yield grade of the carcass. These authors developed the modified on-line altered suspension (MOLAS) technique which first severs the pelvic bone at the narrowest part of the body of the ilium (Aalhus et al., 1999). An additional two cuts are made to separate the vertebrae and connective tissue between the 6th/7th thoracic and 5th/6th lumbar vertebrae. This allows the LTL to support additional weight from the carcass. Aalhus et al. (1999) evaluated this technique on tenderness of the LTL and inside round and found a marked increase in tenderness without damaging or degrading smaller contralateral muscles. Further work by Shanks et al. (2002) reported greater decreases in shear force values with stretched carcasses for the *semimembranosus* and *rectus femoris* muscles versus muscles from non-skeletal separated carcasses. In contrast, the psoas major developed shortened sarcomere lengths and an increase in shear force. Mandell et al. (2006) reported that the *semimembranosus* was the only muscle in the round that had a decrease in shear force with skeletal separation even though the procedure increased sarcomere length for *semitendinosus*, *semimembranosus*, *rectus femoris*, and *vastus lateralis*. In addition to decreasing shear force values for specific muscles, the use of the stretching technique reduces sarcomere shrinkage associated with rapid cooling in commercial abattoirs. This is important as rapid chilling decreases sarcomere length to a greater extent when stretching or electrical stimulation techniques have not been administered to accelerate the rigor process (Rosenvold et al., 2008). The Tendercut or the Tenderstretch procedures

may both qualify as effective solutions to improve or ensure beef tenderness without the need for altering existing infrastructure in commercial abattoirs. Cargill Meats Solutions beef plants use a modification of the Tendercut procedure in their plants by simply cutting the chine bone at the grading site for both USDA and CBGA grading.

The use of Tenderstretch (altered suspension) and Tendercut (12th thoracic vertebrae severed) procedures are well documented for decreasing the amount of cold shortening in muscle sarcomeres (Hostetler et al., 1972; Claus et al., 1997; Eikelenboom et al., 1998; Aalhus et al., 1999). The Tendercut and Tenderstretch procedures improved *longissimus* tenderness under rapid chilling conditions which predisposes the carcass to cold shortening; however skeletal separation procedures did not improve beef tenderness with slower rates of chilling (Hostetler et al., 1972). Due to the elongation of the stretched muscles with skeletal separation, fabrication of the muscle will produce larger numbers of steaks which are smaller than steaks fabricated from muscles of beef carcasses that have been conventionally suspended without use of skeletal separation procedures. This could result in a pricing discount for the smaller size steaks (Soerheim et al., 2001). Soerheim et al. (2001) then suggested that although altered cuts from stretched muscles may not be full size, this concern becomes less pertinent as the demand from consumers changes to more uniform and smaller pre-packaged retail portions (Soerheim and Hildrum, 2002). The use of carcass stretching techniques to isolate “high eating quality” muscles from cull cows and cows of advanced age may increase markets for cow beef and increase returns for the beef industry provided a tender product is assured.

2.1.8 MOISTURE ENHANCEMENT

Moisture enhancement is a process by which a fresh meat product is marinated, vacuum tumbled, or commonly an injection marination of a solution containing specific ingredients (water, salt, phosphates, enzymes, and other organic acids) to increase the palatability or organoleptic properties. Postmortem use of injection marination (moisture enhancement) has been well established (Aktas and Kaya, 2001; Baublits et al., 2005; Cheng and Ockerman, 2007) for dealing with the inconsistencies in tenderness, juiciness, and flavor from middle-aged cows. Moisture enhanced product is currently marketed as “seasoned” beef. Extensive research has found moisture enhancement to increase tenderness in many beef muscles such as *longissimus* and various muscles in the round (Alarcon-Rojo and Dransfield, 1989; Hall Jr., 1995; Thomson and Dobbie, 1997; Berge et al., 2001). Solution marination (Moisture Enhancement) has also been carried out for many decades to improve organoleptic properties, including juiciness and flavour of beef to acceptable or enhanced levels (Brotsky, 1976; Smith et al., 1984; Molins et al., 1987; Papadopoulos et al., 1991c; Craig et al., 1996; DeYonge-Freeman et al., 2000; Lawrence et al., 2003a; Maca et al., 1997). Using many types of injection methods, the use of solutions containing sodium or calcium (Ca) phosphates, lactate, or chloride solutions may improve product quality and consistency (Vote et al., 2000; Lawrence et al., 2004; Baublits et al., 2006). Moisture enhancement can also increase overall moisture yield and weight creating a value added product.

Sodium, calcium and/or phosphate solutions have been used to enhance moisture, water-binding ability, and/or palatability traits in beef (Vote et al., 2000; Lawrence et al.,

2003b; Lennon et al., 2006). Sodium phosphate solutions increase water holding capacity by concomitant increases in muscle pH (Bendall, 1954). Moisture enhancement is widely used in the pork industry, utilizing variable solutions based on water, sodium phosphate and sodium chloride to enhance and (or) maintain tenderness, juiciness, and flavour of lean pork cuts (Vote et al., 2000). While injection technology has been adopted for some beef products, Vote et al. (2006) reported improvements in tenderness, juiciness and cooked beef flavour by injecting subprimal beef strip loins with sodium phosphate, sodium lactate, or sodium chloride solutions. Use of sodium tripolyphosphate alone for moisture enhancement failed to increase tenderness and imparted a sour or soapy aftertaste. Moisture enhancement techniques employing Ca based injections should, in theory, further increase tenderness. The use of Ca to enhance myofibrillar structures has been investigated by many researchers to decrease shear force values and increase overall tenderness (Morgan et al., 1991; Diles et al., 1994; Lawrence et al., 2003a; Baohua Kong et al., 2006). Calcium based solutions are incorporated to increase tenderness by providing Ca^{2+} ions to increase calpain activity during muscle conditioning (Whipple and Koohmaraie, 1993; Geesink et al., 2006), thereby weakening the myofibrillar structures as well as collagen cross-linkages when aged. However, infusion of calcium ions 0.5-6 h postmortem increased tenderness; this was attributed to the reduction in cold-shortening through acceleration of postmortem glycolysis rather than activation of the calpains (Rees et al., 2002).

Calcium lactate or lactic acid injections have been investigated for their influence on colour stability, sensory properties, and tenderness of beef (Aktas and Kaya, 2001; Berge et al., 2001). The injection of the lactate ion was believed to promote colour

stability and may address both safety and quality issues (Seyfert et al., 2007). Other research (Lawrence et al., 2004; Mancini et al., 2005; Kim et al., 2006) measured potassium lactate on meat colour chemistry and has reported an increased amount of lactate dehydrogenase (LDH) activity, metmyoglobin-reducing activity (MRA) and colour stability during a 7 d display (Seyfert et al., 2007). Aktas and Kaya (2001) found brown coloured steaks were produced when steaks were marinated with lactic acid; the marinade apparently enhanced oxidation of myoglobin to metmyoglobin with lower colour intensity when compared to control steaks. The injection of 0.5 M lactic acid into collagen-rich beef *M. pectoralis profundus* muscle markedly reduced toughness of meat as soon as 2 d postmortem (Berge et al., 2001), suggesting that both myofibrillar toughness and, to a lesser extent, the background toughness were decreased. It is worth noting that development of a sour flavour may occur when meat pH drops below 5.0 (Eilers et al., 1994). Marination of meat in lactic acid solutions greater than 1.0% produced a sour taste which was less acceptable than control steaks or steaks moisture enhanced using either sodium or calcium chloride solutions. Use of chloride solutions, including calcium chloride solutions, were effective for increasing tenderness in beef from mature cows (Hoffman, 2006) and accelerating tenderization postmortem in lamb and Brahman-cross bred steers (Koohmaraie et al., 1990). Hoffman (2006) examined use of moisture enhancement for *semitendinosus* and *longissimus* muscles from mature Simmental cows ranging from 10-13 yr of age. Injection of commercial salt mixtures yielded increases in tenderness scores and salty flavour when evaluated by trained sensory panellists.

As previously stated, moisture enhancement is used to increase juiciness for the

consumer. Injection of meat with salt based solutions allows for a larger interfilament swelling to occur by increasing the negative charges as well as the salt effects on the cross links between actin and myosin (Offer and Trinick, 1983). This acts to increase overall water holding capacity for the injected muscle. The use of phosphates in commercial injection processing increases the water-binding ability of meat samples; however, Lawrence et al. (2004) suggests that phosphates can chelate Ca in solution rendering the free phosphates incapable of being effectively used in marination brines. However, since muscle proteins are already bound to Ca, the added phosphates will not complex with Ca, allowing them to be free and available to operate in a moisture enhancement solution (Inklaar and Fortuin, 1969). The chloride ions in salt/phosphate solutions may increase electrostatic repulsive forces between themselves and myofilaments (Offer et al., 1984). These repulsive forces allow water to fill into the interfilament spaces being held by the water's capillary forces. Thus, maximum water holding capacity is achieved with a lower amount of chlorine (Hamling and Calkins, 2008). Vote et al. (2000) examined use of sodium tripolyphosphate, sodium lactate, and sodium chloride for moisture enhancement. Use of sodium tripolyphosphate alone did not appear to increase tenderness and juiciness while decreasing flavor intensity and imparting an off-flavour characterized by sensory panellists as soapy and sour. Moisture enhancement of *longissimus* muscle with sodium chloride and Ca chloride increased tenderness, juiciness and flavor (Aktas and Kaya, 2001) in approximately 2 yr old steers. These researchers also used lactic and citric acids for moisture enhancement to increase tenderness and breakdown of myofibrillar proteins through ageing. Use of sodium chloride and Ca chloride for moisture enhancement did not contribute to any increased

myofibrillar breakdown. Lawrence et al. (2003) examined various moisture enhancing treatments involving Ca to determine their effects on tenderness and retail life span for beef *longissimus* muscle; this included a simulated retail display for 5 d and to evaluate product color on 24 h increments for L* a* b* values. Purge losses were greater for all Ca treatments than non-moisture enhanced, control group and muscles injected with distilled water even though the researchers initially hypothesized that the distilled water group would have the most purge loss due to the weakest presence of ionic bonding strength of moisture within the meat. Distilled water injection had the lowest pump % yield; however after retail display, the distilled water injected samples had the least colour deterioration.

Lactic and ascorbic acids have been used for moisture enhancement of beef *longissimus* to combat the variability in beef tenderness, juiciness, flavour, and retail color. Lactic acid has been traditionally used for softening and flavouring of meats by decreasing mechanical resistance of meats, including muscles that have high connective tissue content (Kijowski and Mast, 1993). Using an injection device, the limitation of treatment efficiency due to slow penetration of exogenous acids is minimized and allows more rapid diffusion of the acid solution into the targeted muscle, increasing tenderness and decreasing mechanical strength. This is believed to be accomplished through lactic acid's ability to weaken myofibrillar and connective tissue structure (Berge et al., 2001). Lawrence et al. (2003) found that using calcium lactate solutions (0.1 - 0.3 M) for moisture enhancement increased softness and tenderness compared to simple injection of distilled water. The benefits of tenderization with lactic acid were also investigated for effects on colour stability. A recent study with ground beef found that metmyoglobin-

reducing activity is increased with sodium, calcium, or potassium lactates; however, none of the treatments increased color stability (Seyfert et al., 2007). Perhaps more research is needed to test the effects of lactic acid on cull cow beef to confirm these results before discounting lactic acids for moisture enhancement.

Ascorbic acid, also known as vitamin C, has also been used to improve tenderness, lipid stability and retail shelf life in many beef products (Wheeler et al., 1996; Sanchez-Escalante et al., 2001; Lawrence et al., 2003a). From the conversion of muscle to meat throughout retail display, meat is subjected to microbial and chemical deterioration that inevitably reduces palatability, appearance and other organoleptic properties. Wheeler et al. (1996) examined the anti-oxidative properties of Ca ascorbate in combination with Ca chloride (CaCl_2) and found ascorbic acid can be added to CaCl_2 injection brines to offset potential colour deterioration in beef after 5 d of retail display due to salt-induced oxidation. Ascorbic acid was also injected into the *longissimus* muscle and had increased colour stability. To minimize spoilage and microbial growth, Sanchez-Escalante (2001) examined the effects of using ascorbic acid on beef patties in a modified atmosphere, a common way to extend shelf-life for oxygen sensitive red meats. Ascorbic acid alone or in combination with carnosine (a potent anti-oxidant) was effective in inhibiting lipid oxidation as assessed using TBARS and myoglobin assays. However, Lawrence et al. (2003) discovered that using Ca ascorbate at higher than 0.2M concentrations may have contributed to a tenderization effect but at the expense of higher incidence of off-flavour and an increase in discoloration. The Ca ascorbate treated steaks were darker, more brown (higher hue angle) and were less red than steaks moisture enhanced with Ca lactate or Ca chloride (Lawrence et al., 2003a); these findings

contradicted the work of Wheeler et al. (1996) using Ca ascorbate in combination with CaCl_2 . Furthermore, Ca ascorbate has been used to replace sodium chloride in fermented sausages, increasing a^* and b^* values while decreasing L^* values vs. control steaks (Gimeno et al., 2001). The use of Ca ascorbate for moisture enhancement improved tenderness, offset colour deterioration and increased colour stability in past studies; however there is little information on consumer palatability or if any flavour variation has been detected. Lawrence et al. (2003) conducted a sensory evaluation with trained panellists to describe off-flavours involved with treatments including Ca ascorbate, Ca chloride and Ca lactate. All treatments imparted an off-flavour marked as metallic, soapy or sour when concentrations peaked at 0.3M and decreased with lower concentrations of injected solution. Use of 0.3M Ca Chloride resulted in the most off-flavour followed by 0.3M Ca ascorbate and 0.2M Ca chloride. Past work using *longissimus* muscle moisture enhanced with various concentrations of CaCl_2 and water (Wheeler et al., 1993) found 0.2M CaCl_2 to produce the most desirable product for sensory panellists in terms of juiciness and flavour while water injected muscles had weaker flavour intensity than non-injected controls. Furthermore, tenderness was not significantly different between non-injected and water injected muscles. No other studies have further expanded on the recommendation by Lawrence et al. (2003) to not exceed 0.2M Ca ascorbate for moisture enhancement to avoid problems with colour stability and off-flavours. Additional investigations can be conducted at the recommended concentration levels for Ca chloride, Ca lactate, and Ca ascorbate to better understand the color, texture, tenderness, and flavour attributes imparted by moisture enhancement.

Desirable beef colour for consumers may be an issue with the introduction of

injected solutions (pumping) into whole beef muscles to moisture enhance the product before fabrication into retail cuts. Lawrence et al. (2003) acknowledged that some moisture enhancement agents such as calcium ascorbate decrease shelf life due to extensive discolouration. Contrary to their hypothesis that stated calcium ascorbate may sequester the free radical electrons to stabilize oxidation and reduce the formation of metmyoglobin and carboxymyoglobin, moisture enhancement of *longissimus* muscle with calcium ascorbate decreased L* values, causing darkening of injected meat. Conversely, Wheeler et al. (1996) found that enhancement with ascorbic acid had a positive effect for oxidative stability and shelf-life with most subprimal cuts. Ascorbic acid can also be combined with CaCl₂ to stabilize oxidation of myoglobin and thus extend shelf life as well as enhance tenderness (Wheeler et al., 1996). A limited amount of research has been conducted solely based on calcium ascorbate and its effect on colour stability and instrumental values to assess colour whether using objective grading or colorimetric techniques. Moisture enhancement of *longissimus* with 0.2 M Calcium ascorbate caused significant discolouration when compared to a non-injected control, or moisture enhancement with water, calcium chloride or calcium lactate (Lawrence et al., 2003b). Concentrations of calcium ascorbate or calcium lactate above 0.1 M may increase colour augmentation; particularly darkening meat colour. The changes in a* values found using calcium ascorbate for moisture enhancement may be due to action of calcium ascorbate in the presence of copper or iron; calcium ascorbate can act as a pro-oxidant, increasing lipid oxidation and discolouration (Lawrence et al., 2003). Use of 0.2 M Calcium ascorbate for moisture enhancement (11% by weight) may improve tenderness but at the expense of lipid oxidation and/or colour instability.

Although studies involving general meat lipid oxidation are plentiful, little research has been published regarding calcium ascorbate and its effects on microbial growth for food safety. Use of citric or acetic acid for moisture enhancement decreased microbial growth of *E.coli* O157:H7 when treated with at least 0.25% of the acids when compared to control steaks. However, calcium ascorbate has minimal ability to limit microbial growth according to Mukherjee et al. (2008). Lactic acid and acetic acid were beneficial for reducing the log CFU/cm² counts of salmonella or enterobacteriaceae species (Anderson and Marshall, 1990). While a moisture enhancement agent may control lipid oxidation, injected tenderizers such as calcium ascorbate do not appear to affect aerobic microbial growth. Perhaps further research utilizing calcium ascorbate as a moisture enhancement can provide greater depth of knowledge on its effects on microbial growth and ultimately its relevance to food safety. The use of calcium lactate for moisture enhancement decreased aerobic bacterial plate counts (cfu/cm²), while there was no effect on final plate counts using calcium ascorbate (Lawrence et al., 2003). In contrast, Wheeler et al. (1993) reported higher total aerobic plate counts marinating with calcium chloride vs. non-marinated controls. Moisture enhancement with brine solutions increased bacterial counts at weekly storage times on pork loins but there were no consistent injection treatment effects on bacterial growth (Bohaychuk and Greer, 2003).

2.1.9 SENSORY EVALUATION OF BEEF

Tenderness as an organoleptic attribute is the single most important trait that influences a consumer's eating experience (Jurie et al., 2007). The perception of eating

quality is a major deciding factor whether a consumer is willing to purchase a particular meat product. Sensory evaluation and (or) mechanical strength tests, such as WBSF can be used to assess tenderness for determining potential eating quality. This standardized procedure is utilized for the comparison or benchmarking of inherent muscle properties in beef throughout the industry (Wheeler et al., 1997b; Stelzleni et al., 2007; Hildrum et al., 2009). The consumer's purchasing-decision process for beef retail cuts not only relies on appearance (colour) but also relies heavily on tenderness, juiciness, and flavour (Carmack et al., 1995). Consistent tenderness and meat quality also play a large role in the decision process when consumers purchase a specific meat product. Boleman et al. (1997) conducted a study to determine how known shear force values affected customers' intent to purchase steaks in higher price categories if tenderness was guaranteed. After consuming the steaks, study participants were willing to pay a premium for improved or an even more tender retail cut. Consumer evaluation of beef products in tandem with shear force determinations are useful means to determine levels of tenderness; however trained sensory evaluation panellists can provide more objective and consistent results when evaluating meat products and their organoleptic attributes (AMSA, 1995). Sensory evaluation is widely accepted and defined as a scientific method used to evoke, measure, analyze and interpret responses to products as perceived through the senses of sight, smell, touch, taste, and hearing (Lawless & Heymann, 1999). To evoke and measure the perceived sense from a particular product, standard procedures are established on how to prepare samples, select possible candidates, and train participants. These standardized procedures aid in minimizing bias and error factors with collected sensory analysis data. Errors such as positional bias (order effect), central tendency errors, halo effects,

carryover, and even motivation can influence a panellist and risk the generation of psychological errors (Post et al., 1991). Careful planning of experimental design, sample tests, and statistical analysis techniques will allow investigators to control these factors as well as give tangible conclusions based on the results that were analyzed.

As with the consumer's purchasing decision, meat quality evaluation by means of sensory evaluation aims primarily to measure tenderness, juiciness, and flavour. The use of a postmortem technique, including moisture enhancement and skeletal separation, may have measurable and beneficial effects on these meat quality characteristics. However, variation in tenderness, juiciness, and flavour between untreated, excised muscles exists. In a study evaluating beef from 8 USDA Select/Choice grade steers, the *semimembranosus* was ranked as one of the most flavourful muscles out of a group of 12 muscles from various primal cuts while at the same time ranking very low for juiciness and tenderness (Carmack et al., 1995). In addition, Carmack et al. (1995) found *semitendinosus* and *longissimus* (striploin) muscles to have much lower beef flavour intensity. While Lawrence et al. (2003) found moisture enhancement with Ca ascorbate to decrease beef flavour intensity when compared to control LM steaks, other researchers reported increases in tenderness with the addition of Ca salts (Lennon et al., 2006), salts and phosphates (Wheeler et al., 1991), or Ca based moisture enhancement treatments (Lawrence et al., 2003a). Juiciness is also a very important positive consumer attribute associated with a pleasant eating experience. With the exception of fresh steaks, many retail steaks are frozen before being cooked. Contrary to what some consumers may believe, it has been found that freezing does not affect muscle juiciness; however the time spent being stored directly effects the water holding capacity as well as the juiciness of

the steak after being cooked (Bouton et al, 1958).

Steaks cut from beef muscles are graded, at least in part, by determination of the amount of subcutaneous, intermuscular and intramuscular fat depots visible. Although grading standards vary by country, American and Canadian grading systems use marbling grade as the major criterion for quality grade, with marbling grades assigned based on the amount of intramuscular fat present in *longissimus* muscle. The appearance of beef muscles and steaks, which is partially influenced by marbling fat presence, is used as a standardized grading scheme to determine either intramuscular visible fat content for marbling or subcutaneous fat for carcass lean scores that reflect a particular meat sample. The overall quality and palatability of a highly marbled steak is expected to provide a more soft, juicy and flavourful steak to consumers than the eating experience provided by a similar but less marbled steak (Killinger et al., 2004). Steaks that have inherently more intramuscular fat (higher quality grade) have also been rated more juicy to similarly but less marbled steaks from the same muscle (Saffle and Bratzler, 1959). Marbling to an average consumer may generally represent a more costly steak which is believed to create a better eating experience, of which consumers have shown interest in paying a premium for (Platter et al., 2005). For the meat processor, marbling grades are important for classifying red branded products to set market values as well as be able to describe product quality to consumers. The amount of marbling fat content within retail cuts provides a manner to produce a price differential and ideally give consumers an idea to what kind of eating quality experience they may encounter. Consumers prefer a more tender, juicy and flavourful product over a less tender, more dry and bland product. For cull cow beef, addressing the eating experience issues for tenderness, juiciness, and

flavor may help increase consumer demand. To do this, sensory evaluation protocols are valuable in determining products and product specifications which will yield organoleptic properties associated with a product that will generate repeat sales by consumers.

2.2.0 CONCLUSIONS

The inconsistent meat quality associated with mature cull cows is an ongoing problem and affects how cull cow beef is utilized in the meat industry. Because cull cow beef is considered less tender than beef from youthful carcasses (steers and heifers), there are limited marketing opportunities which impacts value and returns to producers. Improving the quality and consistency of cull cow beef may not only increase demand for beef products but allow producers to increase value in otherwise discounted product or product destined for further processing. The practice of postmortem ageing for many beef products is widely used as a method to aid in the tenderization of beef primals, muscles and steaks; although for cull cow beef, it alone cannot guarantee a more tender eating experience. The use of carcass stretching techniques (skeletal separation techniques) can increase sarcomere length and subsequently increase tenderness in various muscles, provided the stretching techniques do not require drastic changes to existing abattoir infrastructure. Furthermore, moisture enhancement or injection marination may prove to be a valuable tool for increasing water holding capacity, overall tenderness, juiciness, and lipid oxidation stability in cull cow beef similar to the effects found with steer and heifer beef. The injection of Ca based solutions aids in weakening

of myofibrillar structures via the calpain enzyme system with little or no effect on microbial growth. The calpain system is activated and regulated by Ca^{+2} ion presence, increasing tenderness as beef is aged. Both moisture enhancement and carcass stretching techniques individually have a documented effect on meat tenderization and eating quality of beef. However, to our knowledge no one has investigated combining skeletal separation and moisture enhancement to improve the eating quality of cull cow beef. Evaluating the effects of a modified, skeletal separation processing technique and moisture enhancement via injection processing may help develop processing procedures for cull cow beef to ensure a more consistent and higher quality product which may increase consumer demand and increase returns to producers.

3.1. ABSTRACT – THE EFFECTS OF SKELETAL SEPARATION AND MOISTURE ENHANCEMENT ON EATING QUALITY OF CULL COW BEEF

Sixty-two cull beef cows of known age and breed were slaughtered without electrical stimulation to investigate the effects of skeletal separation and moisture enhancement effects on eating quality. Carcass sides were designated to one of 4 postmortem processing treatments: 1) No additional postmortem processing (NPP), 2) Prerigor skeletal separation (SS) after carcass dressing, 3) Moisture Enhancement (ME) using Calcium Ascorbate (CaAsc) 24 h postmortem and 4) Combination of SS and ME. Skeletal separation involved severing the pelvic bone at the narrowest part of the body of the ilium, and detaching vertebrae and connective tissue between 6th and 7th thoracic and 5th and 6th lumbar vertebrae. At 24 h postmortem, *longissimus* muscle from the ribeye (RE) and loin (LOIN) along with *semimembranosus* (SM) and *semitendinosus* (ST) muscles were excised from each carcass side with designated muscles injected with CaAsc (11% by wt). All muscles were cut into 2.54 cm steaks for subsequent meat quality and characteristics evaluation including colour, pH, sarcomere length, lipid oxidation, aerobic plate count, 4 d retail shelf life simulation, and shear force (SF) determinations of tenderness for 7, 14, and 21 day aged products. A 10 member trained taste panel assessed treatment differences in palatability attributes for 14 d aged RE and ST steaks as well as comparing sensory findings for cull cow vs. red branded beef from AA (USDA Select) and AAA (USDA Choice) quality grade carcasses. Skeletal separation increased sarcomere length and tenderness for the ribeye and strip loin muscles. Moisture enhancement increased tenderness for all muscles compared to that of

the NPP steaks. Although moisture enhancement increased tenderness and was rated higher in juiciness compared to NPP or SS steaks, ME steaks had a higher incidence of off-flavour compared to steaks not injected with CaAsc. Steaks injected with CaAsc tended to be darker, duller, discoloured and were less red and more brownish in colour. Postmortem processing RE steaks were rated by panellists as similar or superior in juiciness and flavour as Canadian grade AA or AAA beef. Postmortem processing ST steaks were rated superior in juiciness and similar or superior in juiciness as Canadian grade AA or AAA beef. Steaks treated with both skeletal separation and moisture enhancement yielded a more tender product than that of any other single postmortem processing treatment. The utilization of both skeletal separation and moisture enhancement via CaAsc solution injection may create a value-added product that is more consistently tender and juicy. This product could be marketed in the fresh meat case as an alternative to marketing cull cow beef as a lower priced, ground beef or processed product.

Key Words: Cull cow beef, Skeletal Separation, Moisture Enhancement, Shear Force, Sensory Evaluation, Eating Quality

3.2. INTRODUCTION

Since the discovery of BSE in 2003, Canadian beef cow-calf and dairy producers have received only 30-80% of past market revenue in comparison to 2002. Although 2007 brought the opening of the Canadian-USA border for beef > 30 mos. of age, cow prices have not consistently returned to the pre-BSE figures (Statistics Canada, 2010). To increase returns for cow-calf producers, cull cow beef needs to be utilized as whole muscle cuts or steaks in the fresh grocery retail trade to be sold at premium prices in contrast to current usage as ground and processed beef (Mandell et al., 2006; Stelzleni et al., 2007). Unfortunately, fundamental barriers exist that limit the usage of cull cow beef to lower quality, lower priced processed meat products; this includes problems with eating quality (tenderness, juiciness, flavour) as well as consistency in tenderness (Xiong et al., 2007). Antemortem processes have been considered to improve cull cow beef quality. This includes feeding higher quality diets or determining proper finishing times for cull cows. However, this can be expensive and may not be cost effective for increasing cull cow beef quality and consistency (Schnell et al., 1997). The use of postmortem processing techniques may be best suited to address quality issues for cull cow beef by creating value-added products that could be sold at a market premium. This would increase prices for cull cows and returns to cow-calf producers. Investigation into postmortem processing to enhance the quality of cull cow beef may be cost effective to create higher quality, value-added products.

There are various postmortem processing techniques available for the beef processing and packing industry. These include the use of skeletal separation techniques

where select cuts are made on carcasses designated to stretch muscles and ultimately increase tenderness. The select cutting of vertebrae, connective tissue, fat, and minor muscles around major muscles of interest has been examined on beef carcasses for over 20 years (Beatty et al., 1999). The goal of the procedure is to increase sarcomere length by increasing tension on specific muscles by the suspended weight of the carcass. The increase in sarcomere length has been correlated in the past with increases in tenderness and decreases in shear force in the *longissimus* and select muscles in the round (Ludwig et al., 1997; Claus et al., 1997; Shanks et al., 2002). Claus et al. (1997) reported the use of skeletal separation reduced the variation in tenderness of the *longissimus* muscle in comparison to non-treated carcass sides. The use of similar carcass skeletal separation techniques could aid in increasing tenderness of cow muscles from the *longissimus* area as well as cow muscles from the round that are currently only utilized for processed beef (deli meats and heat and eat roasts) instead of stand-alone retail cuts due to their inherent toughness.

Another post-mortem processing technique is moisture enhancement where beef cuts are injected or marinated with a food grade chemical or marination solution to increase eating quality attributes including tenderness, juiciness, and flavour. Moisture enhancement has been carried out for the past 30 years to improve the eating quality of whole muscle and retail cuts from the chuck and round of beef carcasses. Using injection or marination, moisture enhancement solutions including commercial salts, phosphates, calcium salts, and weak organic acids have shown promise for increasing tenderness in mature beef (Baohua et al., 2006; Lawrence et al., 2003b) as well as improving textural properties including shear force, tensile strength, and sensory tenderness scores for beef

from youthful carcasses (Berge et al., 2001; Baublits et al., 2006). However, a major problem with injection of calcium chlorides and calcium lactate is the tendency for the solutions to create accelerated lipid oxidation, off-flavours or colour deterioration over typical retail shelf life (Lawrence et al., 2003a). While injection of beef strip loins with solutions containing sodium tripolyphosphate, sodium lactate, and sodium chloride did increase tenderness, off-flavour ratings increased as well as salty comments from panellists (Vote et al., 2000). While calcium lactate imparts off-flavours, substitution with calcium chloride also improved water binding ability to create a juicier product. However, when phosphates were added in tandem with calcium chloride, the phosphate chelated with the calcium in solution preventing their benefits from occurring in a single solution. (Lawrence et al., 2003a) Lawrence et al. (2003b) also found that staged marination can improve the tenderness and water holding capacity if injection of calcium lactate, phosphate, and salt occurs at different times postmortem. The use of calcium to activate the calpain proteolytic enzyme system for increased tenderness in beef is well established (Whipple and Koohmaraie, 1993; Wheeler et al., 1997; Lawrence et al., 2003a). However, colour deterioration and lipid oxidation have been ongoing problems with moisture enhanced beef product and methods need to be developed to curb these effects on retail shelf life. Studies using ascorbic acid have shown potential colour stabilizing effects for pork and other beef products including sausages (Gimeno et al., 2001; Sanchez-escalante et al., 2001). The use of calcium ascorbate was investigated on beef strip loins from youthful cattle and has shown potential for increasing tenderness and inhibiting lipid oxidation; however, use of calcium ascorbate also increased off-flavours when injecting a 0.2 M calcium ascorbate solution at approximately 11% by

weight. There is potential for calcium ascorbate to be used as a tenderizer and colour stabilizer if more was known on its effects on sensory attributes. Additional research is merited to determine the effects of moisture enhancing cull cow beef with calcium ascorbate to determine if the benefits are present and if excessive off-flavours may be detected after the cooking process.

To date, no one has investigated both skeletal separation and moisture enhancement together to determine whether the individual techniques and their effects are still present or if a potential additive effect may occur. The objectives of this investigation are to examine the effects of skeletal separation and/or moisture enhancement using calcium ascorbate on various parameters. This includes:

- 1) the eating quality (tenderness, juiciness, flavour) of cull cow beef,
- 2) pH, and product colour,
- 3) carcass measures and sarcomere lengths to determine the effectiveness of skeletal separation for stretching various muscles from the carcass,
- 4) product colour, pH, and lipid oxidation throughout a 4 d shelf life study
- 5) the eating quality of the cull cow beef products compared with red branded Canadian grade AA and AAA graded steaks.

The ratings generated by the panellists will help determine whether the skeletal separation and/or moisture enhancement techniques can create a value-added product that would be comparable to the red branded beef found in retail stores. Success with these postmortem processing techniques on cull cow beef could increase prices for cull cow and returns to cow-calf producers.

3.3. MATERIALS AND METHODS

3.3.1 ANIMAL PROCESSING

Sixty two cull beef cows of either predominantly British or Continental breeding were sourced from the University of Guelph's, Elora and New Liskeard Beef Research Stations. The cows were culled from the herd due to being open or having bad temperament and(or) poor feet or udders. The cows had been previously managed at their respective research station as part of the breeding herd according to the protocol approved by the University of Guelph Animal Care Committee, based on guidelines and principles of the Canadian Council on Animal Care (Olfert et al., 1993). The cull beef cows aged from 3-12 yrs. were slaughtered at the University of Guelph Meat Laboratory under CFIA inspection according to industry procedures that included stunning with captive bolt prior to exsanguination. Cows were slaughtered primarily in groups of 4 to 6 head per day. Between 45-60 min postmortem, carcasses were split in half longitudinally with each side individually tagged and side weights recorded. The overall length of each carcass side was measured from the top of the rail to the most anterior cervical vertebra with measurements recorded. For individual carcasses, each side was allocated to be either chilled intact at 1.5°C for 24 h prior to processing to undergo a skeletal separation procedure on the kill floor following the modified online altered suspension (MOLAS) procedure of Aalhus et al. (1999). In the MOLAS procedure, the pelvic bone is severed with a bone saw at the narrowest part of the body of the ilium, followed by two more separating cuts to detach the vertebra and all connective tissue between the 6th and 7th

thoracic vertebrae and the 5th and 6th lumbar vertebrae. This results in the *Longissimus et lumborum* (LTL) supporting most of the forequarter weight of the carcass. A second measure of carcass length was conducted on MOLAS-treated carcasses immediately after skeletal separation with measurements recorded prior to overnight chilling of the carcass as previously described.

Twenty-four hours after slaughter, carcass length was measured on all carcass sides again with values recorded. Carcass sides were divided at the Canadian grading site for beef between the 12th and 13th ribs, and assessed for grade fat (G) (mm; minimum fat depth in the last quadrant over the *longissimus* muscle), *longissimus* muscle area (LMA) (cm²; the LM was traced on acetate paper and LMA estimated using a modular system for quantitative digital image analysis [MOP-3, Carl Zeiss Inc, Montreal, Canada]), and subjective marbling score based on marbling grades for youthful cattle: (0 = Devoid; 1 = Grade A; 2 = Grade AA; 3 = Grade AAA; 4 = Prime) with intramuscular fat content being lowest in devoid to a fairly abundant amount of intramuscular fat in prime cuts (CBGA, 2010). Four muscles were then excised per carcass side: *longissimus et thoracis* from the forequarter to produce the rib eye (RE), *longissimus et lumborum* from the hindquarter to produce the strip loin (LOIN), the *semitendinosus* (ST), and the *semimembranosus* (SM). All muscles were trimmed of excess fat, connective tissue, and adjoining muscles, and then labeled with animal ID, treatment, and muscle identification. An attempt was made to evenly allocate individual animals to one of 12 possible treatment combinations on a given kill day based on 4 experimental postmortem processing treatments and carcass side (Table 1). The four postmortem processing treatments included: No postmortem processing performed (NPP) which would serve as a

control, the skeletal separation procedure (SS), moisture enhancement with calcium ascorbate (ME), and a combination of both the skeletal separation and moisture enhancement with calcium ascorbate (SS/ME). Muscles designated for moisture enhancement were weighed and then injected with an approximately 11% pump rate with 0.2M calcium ascorbate using an Inject Star multi-needle injector (Inject Star-New Twist, Germany). Immediately after injection muscles were reweighed, cut into seven to eight 2.54 cm thick steaks depending on the cut/muscle and tagged. Individual steaks were allocated for analysis of specific meat quality attributes in accordance to Table 2. Non-moisture enhanced muscles were also cut into steaks as previously described. All steaks were vacuum sealed and aged for 7, 14, or 21 days with the exception of 1 day aged steaks evaluated for color and pH. These steaks were cut and placed on butcher paper with the fresh cut surface exposed to atmospheric oxygen in air for at least 60 min to enhance colour development. A 10 g subsample of meat was removed from the corner of each steak used for sarcomere length (Table 2); this sample was placed into a sealable Whirl-Pak bag and frozen at -24°C until analysis.

Colour measurements were obtained from 6 locations on each steak designated for colour evaluation using a handheld Konica Minolta Chroma Meter (Model CR 400; Konica Minolta Sensing Inc., Ramsey, NJ) with illuminant D65 and a 0° viewing angle. Colour data was collected according to the Commission International de l'Eclairage (CIE 1976) L* a* b* scale with: L*, a measure of luminosity with a higher value being indicative of a lighter color, a*, a measure of the continuum from red to green, and b*, a measure of the continuum from yellow to blue. For a* and b* measurements, higher positive values indicate a more intense red or yellow colour respectively, while negative

values indicate respectively a more green or blue colour. The colorimeter was standardized with a white ceramic tile with specifications of $Y = 92.7$, $x = 0.3134$, and $y = 0.3196$ (McGregor, 2008).

Temperature and pH were measured using a Hanna spear tipped pH electrode (Hanna Instruments, Mississauga, ON) and thermocouple attached to an Accumet A71 pH meter (Fisher Scientific, Toronto, ON). The pH meter was allowed to equilibrate to the processing room temperature and calibrated with buffer solutions (pH of 4 and 7) prior to taking measurements. Colour, pH, and temperature data were also collected on 14 day aged steaks using the methods previously described. A shelf life study was conducted with 14 day aged steaks which were weighed and placed on 17S Styrofoam® trays with soaker pads (Shortreed, Guelph, ON) and then overwrapped with an oxygen permeable commercial polyvinyl chloride (PVC) film (Resinite, RMF 61-Hy, 1400 cc O₂/100 in²/24 h at 23 °C). These steaks were then placed in a cooler at 1.5°C for 4 days in order to simulate retail display. Steaks were then weighed back for determination of display losses (% Display Loss = (Weight prior to display – Weight after display) / Weight prior to display)*100). RE and ST steaks were then processed for determination of aerobic plate counts (method to be described later). All shelf life steaks were evaluated for pH, temperature and objective colour as described previously and then vacuum packaged and frozen at -24°C until processed for TBARS analysis (method to be described later).

3.3.2 LABORATORY ANALYSIS

3.3.2.1 SHEAR FORCE AND COOK LOSS DETERMINATION

Designated steaks (Table 2) were removed from the -24°C freezer and thawed in a 1.5°C cooler for 48 hours prior to cooking. Post-thawing, steaks were weighed (g) and cooked using a Garland Grill (ED-30B; Garland Commercial Ranges LTD, Mississauga, ON). The steaks were cooked to an internal temperature of 70 °C with product temperature continually monitored by a Type K flexible high temperature thermocouple inserted into the geometric center of the steak. Steaks were flipped when they reached an internal temperature of 40°C. Immediately following the removal of the steak from the grill, endpoint temperature and a final weight (g) were recorded. Cook loss was determined by using cooked and raw weights ($\% \text{ Cook loss} = ((\text{Raw weight} - \text{Cooked weight}) / \text{Raw weight}) * 100$). Post-weighing, steaks were placed into polyethylene bags and immediately immersed in an ice bath to stop the cooking process abruptly. Steaks were cooled overnight in a 1.5 °C cooler. Following overnight storage, steaks were allowed to equilibrate to room temperature and approximately eight, 1.5 cm cores were removed from each steak parallel to the muscle fibres (AMSA, 1995) using a drill press mounted corer (Fisher Scientific, Toronto Ontario). Cores were sheared perpendicular to the muscle fibers using a TA-XT Plus Texture Analyzer (Texture Technologies Corp., Scarsdale, NY) fitted with a Warner-Bratzler blade using a crosshead speed of 3.3 mm s⁻¹. Each sheared core produced a peak shear force value using a pre-programmed macro, and all sheared cores were averaged to yield a shear force value for each individual steak.

Pre-programmed macros were utilized in a Sun Microsystems' Texture Exponent program for Windows XP. Shear Force values were determined for all 8 cores per sample and then averaged to determine the peak shear force in kg. Outliers were removed based upon a calculated ratio (Numerically Lowest Shear Value – Mean Shear Force Value)/ 2 times the standard deviation of mean shear force from sample cores).

3.3.2.2 AEROBIC PLATE COUNT ANALYSIS

Aerobic Plate Count analysis was conducted on posterior ribeye (RE) and *semitendinosus* (ST) steaks from the shelf life study based on modification of the methods described by Lawrence et al. (2003) and Gill et al. (2005). Post-completion of the 4-day simulated retail display in the shelf life study, steaks were swabbed using a pre-moistened cellulose acetate microbial sponge (Whirl-Pak ®, Nasco Plastics, WI) on both the top and bottom surfaces of the meat using a 10 cm² plastic template. Sponges were pre-moistened with 10 ml of sterile 0.1% peptone solution prepared according to Gill et al. (2005). After swabbing, sponges were placed back into the Whirl-Pak bags and 90 ml of 0.1% peptone was then added to standardize the volume. Whirl-Pak bags were then shaken and kneaded for at least 2 min to release bacteria from the sponge to the peptone solution. Sponges were further kneaded to remove excess contaminated peptone and removed from bags to be discarded. Bags were then shaken for an additional two min. One ml of sample was removed from each bag and pipetted into screw cap tubes that contained 9 ml of sterile peptone solution; these were then vortexed for 30 s using a Fisher Vortex Genie (Fisher Scientific, Bohemia, NY). From each tube, 1 ml of diluted

sample was then pipetted on the center of Petrifilm Aerobic Count (APC) plates in duplicate following the procedure outlined by 3 M Canada. (3 M Canada, Inc., London, ON). APC plates were then incubated (Isotemp Compact Incubator, Fisher Scientific, Toronto, ON) at 35°C for 48 h. APC plates were then removed from the incubator and the colonies present were counted manually using a handheld counter and recorded. The recorded colonies or colony forming units (CFU) from the APC plates were expressed as log colony forming units per cm².

3.3.2.3 THIOBARBITURIC ACID REACTIVE SUBSTANCES

Sample and solution preparations for TBARS were adapted from procedures described by Wu and Squires (1997) and Squires et al. (1991). Steaks were removed from the -24°C freezer and then placed in a 1.5 °C cooler for 24 h. The steaks were cored randomly throughout the meat using a 5 mm corer to obtain an 8 g representative sample. Cored samples were weighed into a 50 ml blue cap centrifuge tube to which 10 ml of 5% (wt/vol) trichloroacetic acid and 1 ml of (0.5g/l) butylated hydroxytoluene (BHT) in methanol were added. Samples were homogenized using a handheld UltraTurrax T18 homogenizer (IKA Works INC., Wilmington, NC) at 5000 rpm for 30 s and then centrifuged using a Sorvall Legend RT+ Centrifuge (Thermo Electron Corp., Germany) for 10 min at 10°C. In a screw cap tube, 2 ml of supernatant were pipetted from the centrifuged samples and added to 1.0 ml of 0.2% (wt/vol) thiobarbituric acid (TBA) in water. This solution was then heated in a water bath at 85°C for approximately 30 min and then cooled under tap water to room temperature. Sample absorbance was read using

a Shimadzu UV-VIS spectrophotometer (Shimadzu Corporation, Kyoto Japan) at 532 nm (Squires et al., 1991). Absorbance values were then plotted against the standard concentrations of MDA (ng MDA) equivalents present. A stock solution of MDA was prepared by adding 73.2 mg (80 μ l; 1 ml = 0.92 g) of TEP (1,1,3,3-tetraethoxypropane) to 10 ml of 0.1 N HCL in a screw cap tube and then heating the tube for 5 min in boiling water. The stock MDA solution (2.39 μ g/ml) was further diluted to obtain a final working MDA standard solution of 2.39 μ g/ml. All samples were recorded and analyzed in ng MDA/g of meat sample.

3.3.2.4 SARCOMERE LENGTH

Steak samples that were allocated for sarcomere length were thawed for 24 h in a 1.5°C cooler. Samples were then cut parallel to the muscle fibres. Approximately 2 g of each sample was put into 50 ml centrifuge tubes along with 15 ml of buffer. This buffer was previously prepared by adding 0.25M sucrose and 0.02M EDTA into 500 ml of distilled water and stirring on a hotplate until the EDTA is completely dissolved (Aalhus et al., 1999; Janz et al., 2006). The solution was then homogenized with a Polytron homogenizer (Ultra Turrax T18 Basic Fisher Scientific, Toronto, ON) for 10 s at 5000 rpm. One drop of the homogenate was then placed on a microscope slide using a Pasteur pipette and observed through a Carl Zeiss Axioscope compound microscope (Carl Zeiss, Germany) at 100x with an oil immersion lens and an ocular piece at 2.5x. A slide measurement calibration was then completed to determine the length of the field of view as viewed through the microscope. Six pictures were taken with a Canon Rebel XT digital camera (Canon Inc., Tokyo, Japan) and the corresponding images were inputted

into a freeware Image-J program produced by Wayne Rasband at the National Institution of Health (NIH, Bethesda, MD). Sarcomere length (um) measurements were determined on 10 sarcomeres, where one sarcomere is defined as the distance from a designated Z-line to another adjacent Z-line, using the computer's line distance measuring tool. The 10 sarcomere length measurements were then averaged to obtain the average sarcomere length.

3.3.2.5 SENSORY ANALYSIS

Overall sensory analysis and screening and training logistics were adapted from AMSA (1995) and supplemented by materials provided by sensory analysis specialist, Sue Battenham from Sleeman Breweries (Guelph, ON). An email was distributed to the Animal and Poultry Science, Food Science, Human Nutrition, and OVC Biomedical Science departments at the University of Guelph to recruit possible candidates. Inquiring candidates had to consume beef products on a regular basis, have no known food allergies to Ascorbic acid (Vitamin C) and could not be on any salt, caffeine, or sugar restricted diets prior to the study. Twenty candidates were screened for their ability to detect and rank the basic tastes (sweet, sour, salty, bitter, water) and describe specific odours or flavour that they were presented using descriptive words. Ten candidates were then chosen as panellists for the trial and commenced training.

The ten panellists were further trained and tested on their abilities to detect and distinguish between the basic tastes as well as different concentrations of the basic taste solutions. All ten panellists were then trained on how to describe primary, secondary,

and tertiary sensations when they are describing the flavor, feel, and tastes of common foods found in the kitchen. Following these tests, a beef broth was prepared and used to test the panellists on their ability to detect concentration differences and their corresponding flavour intensities. Steaks were sourced from the University of Guelph and used to test an individual panellist's ability to distinguish between tender, intermediate, and tough beef samples. Training began by presenting samples that had extensive as well as easily perceivable differences in tenderness, softness and juiciness. After grasping the concepts of each attribute, panellists were then subjected to incrementally smaller perceivable differences to fine tune their ability to distinguish between each of the beef samples (Meilgaard et al., 1999). Panellists were then introduced to the 10 cm line scale method with verbal intensity descriptors as anchors (Stone and Sidel, 2004) for evaluating meat tenderness, softness, juiciness, rate of breakdown, flavor, and chewiness palatability attributes, and any off-flavours that may be detected.

Cull cows aged between approximately 4-8 yr were selected for two independent trials utilizing the ribeye (RE) steaks for Trial 1 and the *semitendinosus* (ST) steaks for Trial 2 in which all samples were aged for 14 days and then frozen at -24°C. Trial 1 RE steaks were assigned a three digit random number. In addition to the cull cow steaks being evaluated, 14 control RE steaks from each of the Canadian AA and AAA quality grades (CBGA, 2010; red branded beef) were evaluated by the trained taste panel for comparison purposes. These steaks were obtained from youthful heifer and steer carcasses slaughtered at the University of Guelph Meat Laboratory as part of the university's feedlot research program. These red branded steaks were assigned three

digit random numbers and along with the cull cow beef steaks were randomized into groups of six. The same process was applied to Trial 2 evaluating ST steaks, following the completion of the first trial.

For each trial, steaks were thawed in a 1.5°C cooler for 48 h prior to cooking. Steaks were cooked on a Silesia grill (Model: CG-2, Silesia Grill Machines, Oakville, ON) to an internal temperature of 72°C monitored by a Type K flexible high temperature thermocouple which was inserted into the geometric center of the steak. Steaks were then kept warm at 37°C in a Fisher Scientific Incubating oven (Fisher Scientific, Dubuque, IA) for 20 min. Steaks were then cored approximately 20 times with a 12 mm diameter corer; the cores were then placed into 2 oz Dixie cups labeled with their appropriate three digit random number and then the cups were sealed with lids. Samples were put back into the incubating oven until the trained panellists arrived. Panellists were served warmed samples in segregated, white scent-free booths under red lighting and utilized the 10 cm line scale to evaluate the palatability traits including: softness, tenderness, initial juiciness, flavour, off-flavour, overall juiciness, chewiness, and rate of breakdown.

Sensory attributes were evaluated using a scale from 0 to 10 with 0 always representing the left anchor while 10 always represented the right anchor. Sensory attribute definitions and scale values include the following:

1. Softness - the force required to compress the sample between the molar teeth (0 being very firm and 10 being very soft);
2. Tenderness - the force required to chew measured after three chews excluding the

first bite as a chew (0 being very tough and 10 being very tender);

3. Initial Juiciness - amount of moisture released by the sample after five chews (0 being very little and 10 being very much);
4. Flavour - the amount of full meaty flavour present after eight chews (0 being very weak and 10 being very intense);
5. Chewiness - the energy (force plus time) required to chew the sample as it is prepared for swallowing (at nine chews) (0 being very chewy and 10 being not chewy);
6. Overall Juiciness - the overall impression of moistness perceived in the mouth after ten chews (0 being very little and 10 being very much); and
7. Rate of Breakdown - the rate at which the sample disintegrates during the chewing process in preparation for swallowing (0 being very slow and 10 being very rapid).
8. Off flavor –(Described as gamey, grassy, cardboard-like, painty, rancid, unbeef-like) (0 being none and 10 being very strong)

On the 10 cm scale, (0) represented a sample that was extremely firm, tough, dry, weak flavoured or no off-flavour, very chewy and very slow to breakdown whereas a (10) represented a sample that was extremely soft, tender, juicy, intense or strong flavoured, not chewy, and very fast to breakdown.

3.3.3 STATISTICAL ANALYSIS

All data including carcass characteristics, carcass measurements, meat quality evaluation, shelf life, and sensory evaluation data were analyzed using a mixed linear

model within the PROC MIXED procedure of SAS Foundation software (SAS Institute Inc., 2007) based on a 2 x 2 factorial arrangement randomized incomplete block design. The two main effects include skeletal separation and moisture enhancement as postmortem processing treatments with animal serving as the block. Analysis was completed with a determined significance using $P < 0.05$ for all statistical interpretations (main effects and contrasts). The statistical model for carcass characteristics includes date of slaughter, side, postmortem processing treatment, breed (British or Continental), and postmortem processing treatment by breed fixed effects with slaughter age and grade fat as covariates where appropriate. Means separation for postmortem processing treatments were determined using orthogonal contrasts (Kuehl, 2000). Orthogonal contrasts to examine postmortem processing treatments (PPT) included: 1) the comparison of No Postmortem Processing (NPP) vs. all other postmortem treatments (SS, ME, ME/SS), 2) the comparison of SS vs. ME using Ca Ascorbate, and 3) the comparison of the combination of SS and ME using Ca ascorbate (SS/ME) vs. the average of SS and ME using Ca ascorbate (Table 3). Means separation for postmortem processing treatments by breed interactions included: 1) the interaction of No Postmortem Processing (NPP) vs. all other Postmortem Processing Treatments by breed, 2) the Interaction of SS vs. ME by breed, and 3) the Interaction of SS/ME vs. the average of SS and ME treatments by breed (Table 4). The statistical models for carcass drop measurements included date of slaughter, breed, postmortem processing treatment, and postmortem processing treatment by breed interaction as fixed effects. Carcass side and cow were included as random effects. Carcass drop models included slaughter age and grade fat as covariates as well as random statements in which cow was nested in breed

and date of slaughter while carcass side was nested within cow, breed, and date of slaughter. Means separation for postmortem processing treatments and the postmortem processing treatment by breed interactions were determined using orthogonal contrasts previously described.

With the exception of shear force and cook loss data, statistical models for all meat quality evaluation data included postmortem processing treatment, breed, and postmortem processing treatment by breed interaction fixed effects as well as slaughter age and grade fat as covariates. The model included the random effects carcass side and cow in statements identical to those previously described for models evaluating carcass drop measurements. Means-separation for postmortem processing were determined using orthogonal contrasts previously described.

The statistical model for shear force and cook loss data included the fixed effects of date of slaughter, postmortem processing treatment, breed, postmortem processing treatment by breed interaction, postmortem ageing including, postmortem ageing by postmortem processing treatment interactions, postmortem ageing by breed interactions, and postmortem ageing by postmortem processing treatment by breed interactions with the inclusion of slaughter age and grade fat as covariates with carcass side and cow as random effects. Means-separation for postmortem processing treatments and the postmortem processing treatment by breed interactions were determined using orthogonal contrasts as previously described. Orthogonal contrasts to examine postmortem (PM) ageing times for shear force and cook loss data included: 1) 7 vs. 14 and 21 d PM ageing and 2) 14 vs. 21 d PM ageing (Table 5). Orthogonal contrasts were also developed to examine postmortem processing treatments by PM ageing interactions including: 1) No

Postmortem Processing (NPP) vs. all other Postmortem Processing Treatments by 7 vs. 14 and 21 d PM ageing , **2)** No Postmortem Processing (NPP) vs. all other Postmortem Processing Treatments by 14 vs. 21 d PM ageing, **3)** SS vs. ME by 7 vs. 14 and 21 d PM ageing **4)** SS vs. ME by 14 vs. 21 d PM ageing , **5)** SS/ME vs. the average of SS and ME by 7 vs. 14 and 21 d PM ageing and lastly **6)** SS/ME vs. the average of SS and ME by 14 vs. 21 d PM ageing (Table 6).

Statistical analysis for sensory evaluation data for RE and ST trials included evaluation day and treatment*breed fixed effects in the model. The red branded treatments, AA and AAA marbled beef were also added to the model. Random statements included cow nested in date of slaughter and breed, panellist, panellist by treatment interaction, cow by evaluation day nested within breed and date of slaughter. Orthogonal contrasts for sensory evaluation data included: 1) the comparison of No Postmortem Processing (NPP) vs. all other Postmortem Processing Treatments (SS, ME, SS/ME), 2) the comparison of SS vs. ME using Ca Ascorbate, 3) the comparison of the combination of SS and ME using Ca ascorbate (SS/ME) vs. the average of SS and ME using Ca ascorbate, 4) AA and AAA red branded product vs. SS/ME, 5) AA vs AAA red branded product, 6) the interaction of No Postmortem Processing (NPP) vs. all other Postmortem Processing Treatments by breed, 7) the Interaction of SS vs. ME by breed, 8) the Interaction of SS/ME vs. the average of SS and ME treatments by breed, and 9) the comparison of the 2 breeds; British vs. Continental (Table 4).

3.4. RESULTS AND DISCUSSION

3.4.1 CARCASS CHARACTERISTICS

The overall analysis of variance (ANOVA) for carcass characteristics are presented in Appendix Table 1 while the effects of postmortem processing treatment (PPT), breed, and PPT x breed interactions are presented in Tables 7, 8, 9 respectively. Cull cows were randomized across postmortem processing treatments in an attempt to balance the percentage British (BRIT), and Continental (CONT) breeding and average age of cow for the approximately 30-32 carcass sides processed for each PPT. This was not completely accomplished due to the availability of cull cows for a given week of processing as well as the need to change animal allocation on a given day of slaughter to prevent carcasses from being supported by the kill floor if skeletal separation (SS) was applied to extremely large cows. Cows with predominantly British breeding were used less than cows with predominantly Continental breeding for all PPT. There were no differences ($P > 0.12$) in age at slaughter or for any carcass trait across PPT (Table 7) including hot carcass or side weight, measures of back fat at the interface between the 12th and 13th ribs, marbling score, and LMA. However, there was extensive variation in the data set for all carcass traits including 3.2 to 11.9 y of age, 300 to 580 kg HCW, 0 to 27 mm grade fat, 0 to 4 for marbling score, and 64.5 to 138.6 cm² LMA. Past research reported age effects for hot carcass weight and fat thickness with cull cows; hot carcass weight and fat thickness increased with increasing age (Sawyer et al., 2004). While the

present study used cows up to 12 y of age, Morgan et al. (1991) used cull cows aged 12-17 years which had only trace amounts of marbling; further illustrating the carcass characteristic variation through various ages of cull cows. Sawyer et al. (2004) used various slaughter age groups of cull cows in their study and found no age differences in LMA. Age of cull cows, body score/composition and their respective meat quality are generally considered to be strongly heterogeneous (Shemeis et al., 1994). Based on LMA to assess muscling, cull cow carcasses in the present study on average had adequate grade fat to be graded as D1 carcasses (CBGA, 2010). While cull cow carcasses are not normally assessed for marbling, the carcasses had on average adequate marbling to grade as AA marbled carcasses based on criteria used for grading youthful carcasses. Based on the extensive variation in grade fat, the trait was added as a covariate in statistical models where appropriate to account for variation in specific traits.

Although approximately twice as many predominantly CONT carcass sides were used vs. carcass sides from predominantly BRIT cows, age at slaughter, hot carcass and side weights, and mm back fat at the $\frac{1}{4}$ position were similar ($P > 0.12$) between breeds and are evident in Table 8. While measures of back fat at the $\frac{1}{2}$ and $\frac{3}{4}$ positions, grade fat and marbling score were greater ($P < 0.05$) in BRIT vs. CONT cows, LMA was greater ($P < 0.001$) in CONT cows. Due to the significant grade fat differences across the two breeds, grade fat was included in the statistical model to account for the inherent variation. Mature BRIT breed sourced carcass sides were expected to have higher grade fat value, a lower LMA, as well as a lower product yield than that of CONT while CONT animals should have a larger overall mature cow size (Cundiff et al., 1993). The PPT by breed interactions ($P < 0.05$) for hot carcass and side weights (Table 8; Contrast 2) were

due to the varying weights between individual British and Continental animals.

3.4.2 CARCASS LENGTH, CARCASS DROP AND SARCOMERE LENGTH MEASUREMENTS

The overall analysis of variance (ANOVA) for carcass drop measurements are presented in Appendix Table 2 while the effects of postmortem processing on sarcomere length are presented in Appendix Tables 3, 4, 5 respectively. Pre-skeletal separation carcass length was shorter ($P < 0.05$) in control sides with no postmortem processing (NPP) vs. all other postmortem processing treatments (Table 10; Contrast 1) used. This was unexpected as no skeletal separation procedures were applied to any of these sides when initial carcass length was measured. The biological significance of this difference is questionable; this difference most likely represents a wide variation in carcass lengths present across all cull cows. Shorter carcass lengths may be attributed to the use of cows with predominantly British breeding. Post-skeletal separated carcass lengths differed ($P < 0.001$) for all contrasts (Table 10). Carcass length of NPP sides was at least 13.2 cm shorter ($P < 0.001$; Contrast 1) than side lengths for all other PPT. Differences ($P < 0.001$) in carcass length across all PPT may be attributed to the carcass drop 1 h after slaughter for sides undergoing skeletal separation (SS, SS/ME). Postmortem carcass drop measured 1 h after slaughter averaged 18 cm for sides undergoing skeletal separation (SS, ME/SS) versus the 1 cm carcass drop for sides that were not processed postmortem (NPP) or only moisture enhanced (ME). Postmortem processing treatment differences in carcass drop at 24 h were similar to differences found 1 h after slaughter.

In the past, use of skeletal separation procedures on cross-bred steers increased carcass length by up to 10.6 cm versus untreated, control sides (Shanks et al., 2002; Wang et al., 2003). The larger increase in carcass length from skeletal separation in the present study may be due to using a modified version of the Tendercut procedure (MOLAS; Aalhus et al., 2000) with two differently positioned cuts on the cervical and thoracic vertebrae as well as severing the narrowest part of the body of the ilium. The vertebral cuts on the carcasses allowed stretching up to 17 cm 24 h after slaughter. There were no breed differences ($P > 0.74$) for pre- or post-skeletal separation carcass lengths (Table 11) or in carcass drop measurements. In general, there were no PPT by breed interactions ($P > 0.21$) for carcass length or 1 h carcass drop measurements, however, a PPT by breed interaction was present comparing SS vs. ME (Contrast 2; $P < 0.05$) due to a greater change in carcass length between SS and ME sides for BRIT vs. CONT cows. As each PPT did not have the exact same number of BRIT and CONT carcass sides, this difference between treatments is not surprising. Shortening of the carcass sides 24 h postmortem can occur in leaner cows due to cold shortening of myofibrils within muscles that decline in temperature too quickly. This myofibril cold shortening has occurred previously in similar studies (Sorheim et al., 2001; Hildrum et al., 2002) due to muscles that are exposed at the surface of a carcass side and the temperature is below 14-17°C, chilling the muscles quickly causing carcasses to shorten. However, the cull cow carcasses that were utilized in this study had 10.1-13.0 mm of grade fat over the *longissimus* muscle suggesting that the presence of the relatively thick fat cover would insulate the muscle to attenuate the drop in temperature while chilling is completed. Cold shortening should thus not be a major factor in carcass length variation in this study.

Sarcomere lengths for LOIN and RE sections of *longissimus* were increased ($P < 0.001$) using skeletal separation with (or without) moisture enhancement (SS, SS/ME), exceeding sarcomere lengths for NPP or ME ($P < 0.001$; Contrasts 1 and 2). These results agree with previous work (Ludwig et al., 1997; Shanks et al., 2002b), where similar skeletal separation procedures were applied to *longissimus* muscles on carcasses from crossbred steers or heifers. Contrary to previous work completed using similar skeletal separation techniques (Beaty et al., 1999; Shanks et al., 2002b; Mandell et al., 2006), skeletal separation (SS, SS/ME) did not increase ($P > 0.15$) sarcomere length for muscles (SM, ST) from the round. (Shanks et al., 2002a) Shanks et al. (2002a) suggested that muscle fibre orientation in relation to tension contributes to potential sarcomere lengthening. These authors implemented skeletal separation in carcasses from crossbred steers using a variety of methods, including sawing the pelvis at the sirloin-round junction (Tendercut process), separating the pelvic-femur joint, sawing the femur midway between the proximal and distal end, and combinations of these treatments to yield carcass drops of 7.1-10.6 cm. Use of the Tendercut procedure increased sarcomere length for SM vs. controls in the Shanks et al. (2002a) study, while sarcomere length increased for ST only when sawing the femur at the mid-way point. The stretching that occurred on the ST muscle was suggested to be directly attributed to the severing of the femur mid-way. In the present study, the severing of the narrowest part of the body of the ilium was apparently not adequate to stretch the SM and ST; thus an additional cut at the mid-way point on the femur may be adequate to increase sarcomere length for the ST. Sarcomere length was similar ($P < 0.10$) for SS/ME vs the average of SS and ME for both the LOIN and RE. Sarcomere length did not vary across breeds ($P > 0.66$) for any

of the muscles evaluated (Table 11) in the present study (Loin, RE, SM, ST), suggesting that only PPT directly influenced sarcomere length when comparing skeletal separation procedures (SS, SS/ME) to the control (NPP) or ME. Ultimately, the Tendercut procedure was designed for potential increases in sarcomere length for the purpose of increasing tenderness. Creating tension on the specified muscles can prevent shortening during the onset of rigor and these muscles are more tender than that of contracted or shortened muscles (Wang et al., 1994).

3.4.3 COLOUR, PH, MOISTURE ENHANCEMENT, MOISTURE RETENTION AND DRIP LOSS

After moisture enhancement, the LOIN retained approximately 9.0 to 11.6% of CaAsc solution injected by weight (Table 12) while the RE retained approximately 12.4 to 13.2% CaAsc solution (Table 15) injected by weight. Muscles from the round retained less CaAsc solution on average with approximately 9.5-9.9% solution injected by weight retained in SM and 9.0-9.5% solution injected by weight retained in ST (Tables 18 and 21). Lawrence et al. (2003a) reported pump yields of approximately 5.8% when injecting a 0.2M CaAsc solution with a multi-needle injection machine; this is lower than what was achieved in the present study. The LOIN, RE, and SM all retained similar ($P = 0.06$) amounts of CaAsc solution when the respective muscle was obtained from carcasses that underwent skeletal separation (SS/ME) as compared to only moisture enhancement (ME). The trend for increased sarcomere lengths in the LOIN and RE from skeletal separation may create more intercellular space between the actin and myosin components within a

muscle fibre. Since moisture enhancement with salt solutions may aid in disassociating the cross-links between actomyosin postmortem (Lawrie and Ledward, 2006), stretching the muscle fibres via skeletal separation in combination with subsequent moisture enhancement may increase retention of the water-based moisture enhancement solution. The opposite effect was found by Lawrence et al. (2003a) where injection with distilled water decreased water holding capacity by approximately 2.5% due to a decrease in ionic forces within beef muscles. When CaAsc is injected into the muscle, the release of more Ca^{2+} ions may change ion-protein relationships and can alter water holding capacity. This may explain the lower retention of the moisture enhancement solution in the SM and ST where sarcomere lengths were not affected by skeletal separation. Moisture enhancement via injection using sodium tripolyphosphate, sodium chloride (Xiong and Kupski, 1999), and calcium chloride (Aktas and Kaya, 2001) also increased brine retention on a weight by weight basis.

3.4.4 INITIAL DAY OF PROCESSING: LOIN

The overall analysis of variance for initial day of processing, 14 day aged product, and shelf loin for the LOIN is presented in Appendix Table 3. Measurements on the initial day of processing included pH and CIE* colour values with the latter used for calculating hue angle and chroma. Initial day pH was similar ($P = 0.572$) across all PPT (Table 12). On the other hand, Hoffman (2006) found pH to increase by at least 0.3 units after moisture enhancement of strip loin muscles from mature cows using a commercial salt mixture including sodium, phosphate di-triphosphates, lactate and chloride. These

findings were believed to be due to the presence of the lactate and the phosphate ions. Lawrence et al. (2003a) injected strip loins at 0.1 – 0.3 M concentrations using CaAsc and calcium lactate solutions and found no pH differences between the two treatments; however control steaks had a higher pH after marination and ageing. While there were no breed differences ($P > 0.62$) in initial day pH (Table 13), a PPT by breed interaction ($P < 0.03$) was present (Table 14). This was due to lower pH for SS BRIT vs. SS CONT in contrast to a higher pH for ME BRIT vs ME CONT; the biological significance of this interaction is questionable. L^* values did not differ ($P > 0.17$) across PPT (Table 12) in contrast to Lawrence et al. (2003), where moisture enhancement of strip loins using CaAsc darkened the product with lower L^* values. Initial day L^* values were similar ($P > 0.27$) across breeds (Table 13). The use of SS treatments was also investigated by Aalhus et al. (2000) with no differences in lightness between skeletally separated and control carcass sides. The presence of redness or brown discolouration was similar when comparing SS vs. ME steaks ($P = 0.09$; Contrast 2) as well as SS/ME vs. the average of SS and ME ($P = 0.06$; Contrast 3). Previous work (Aalhus et al., 1999) with SS treatments resulted in similar hue angle (similar redness/brownness) to control *longissimus* steaks. A PPT x breed interaction ($P = 0.01$; Contrast 3) was due to smaller differences in hue angle between breeds for SS/ME versus larger hue angle differences between breeds for SS and ME treatments (Table 14). Chroma (colour vividness or intensity) values were greater ($P < 0.01$; Contrast 1) for NPP steaks versus all other PPT. Moisture enhancement decreased ($P < 0.001$; Contrast 2) chroma vs. SS. While not statistically tested, the similar chroma values for NPP and SS contrast to Aalhus et al. (2000) where skeletal separation increased chroma value of strip loin steaks versus non-

treated steaks (Aalhus et al., 2000).

3.4.5 14 DAY AGED PRODUCT: LOIN

The 14 d aged products had a similar pH ($P > 0.30$; Contrast 1) between the NPP steaks and all other PPT. The pH was lower ($P < 0.01$; Contrast 2) for ME vs. SS steaks. As CaAsc is inherently acidic, a decrease in pH would be expected and has been observed in other research when injecting or marinating using weak acids (Berge et al., 2001; Aktas et al., 2003; Lawrence et al., 2003a). The normal ultimate pH range found in beef is typically 5.5-5.7 (Miller, 2007) which encompasses the range in pH across all PPT for 14 d aged product. The variation in pH could be due to the increased acidity created by ME using calcium ascorbate. LOIN pH was similar ($P > 0.81$) across breeds (Table 13). Steaks subjected to ME were lighter ($P = 0.02$; Contrast 2) than SS steaks. Aktas and Kaya (2001) noted the addition of calcium into beef may activate the calpains present, disrupting the myofibrils and creating a paler appearance. This may be responsible for the paler (increased L^* values) product using ME with CaAsc although the values are only marginally greater than the control (NPP). Based on grading standards created by the NPPC, it would be difficult for the human eye to detect these differences in lightness (NPPC, 2000). The NPPC pork colour grades range from 1 (pale pinkish gray) to 6 (dark purplish red); each of the pork colour grades differ from the next ascending or descending numerical pork grade by 6 units for L^* . The SS/ME steaks were lighter ($P = 0.002$; Contrast 3) than the average of the SS and ME steaks and is most likely due to the lower L^* value for SS steaks. Hue angle was higher (more brownish,

less red) in ME steaks than SS steaks ($P = 0.04$; Contrast 2). Hue angle can be used as an indicator of discolouration as it increases in value. Moisture enhancement with CaAsc (ME, SS/ME) increases discolouration over time during aging and retail display, agreeing with Lawrence et al. (2003) when processing strip loin steaks. This is contrary to previous work done suggesting that ascorbic acid can prevent metmyoglobin (MetMb) formation as well as lipid oxidation by acting as an oxygen molecule scavenger restricting pigment oxidation (Renner, 1990). Skeletal Separation is likely not causing an increased hue angle as hue angles were similar between skeletal separation and control product after 6 d ageing (Aalhus et al., 2000). While, there were no breed differences ($P = 0.99$) in hue angle (Table 13), a PPT by breed interaction ($P < 0.02$) was present (Table 14). This was due to hue angle increasing (more brown) ($P = 0.003$; Contrast 2) between SS and ME steaks from BRIT in contrast to a decrease in hue angle (more red) between SS and ME steaks from CONT. The BRIT sourced steaks became more discoloured from ME than CONT sourced steaks. Colour intensity (chroma) tended to decrease with ME with lower values for ME vs. SS ($P = 0.02$; Contrast 2). This agrees with Lawrence et al. (2003a) where colour intensity was greater in control and distilled water injected strip loin steaks as compared to CaAsc injected steaks. Injection of the 0.2M CaAsc solution in the present study tended to decrease colour intensity vs. steaks not subjected to the ME (NPP, SS). Work using culled cross-bred heifer *Biceps femoris* muscles resulted in decreased redness intensity when injecting 0.2 M CaCl_2 (5% w/w) to determine colour changes (Wheeler et al., 1996). In terms of past SS studies, colour intensity of 6 d aged *longissimus* muscle steaks was similar between carcasses undergoing SS and steaks from control carcasses (Aalhus et al., 2000). Chroma values

were similar ($P > 0.14$) between breeds (Table 13). A PPT by breed interaction ($P < 0.04$; Contrast 2) was present with chroma remaining constant between SS and ME steaks from BRIT in contrast to a decrease in chromatic value (colour intensity) for SS vs. ME steaks from CONT (Table 14). In general, a decrease in chroma would represent a decrease in colour intensity and may be less attractive in colour to a consumer looking for a fresh, bright cherry red meat product.

3.4.6 SHELF LIFE DETERMINATION: LOIN

Although pH was lower ($P < 0.04$; Contrast 1) in NPP vs. all other PPT treatments, the difference is of questionable biological significance (Table 12). There were no breed differences ($P = 0.70$) in pH with all values falling within the established “normal pH range” of 5.5-5.7 for beef (Miller, 2007). Lightness (L^*) values for the product were generally similar ($P = 0.08$), suggesting no darkening of the meat after simulated display. As L^* values were similar ($P = 0.06$) in SS vs. ME product, the human eye would most likely not be able to detect this difference as previously described. There were no breed differences ($P > 0.38$) in L^* , hue, or chroma (Table 13). There was no difference ($P > 0.31$) in hue angle across PPT, indicating similar amounts of redness. Chroma was greater ($P < 0.001$; Contrast 1) for NPP vs. all other PPT with lower vividness of the product most likely caused by moisture enhancement as chroma was greater ($P < 0.001$; Contrast 2) in SS vs. ME steaks. The similar values for hue with ME and SS/ME product resulted in lower ($P < 0.04$; Contrast 3) chroma for SS/ME vs. the average of ME and SS steaks. A PPT by breed interaction ($P < 0.03$; Contrast 1) was

present for chroma as there were larger breed differences in the color trait for control product (NPP) vs. breed differences in chroma for all other PPT (Table 14).

Drip loss data are presented using 2 sets of contrasts. Drip loss 1 data follows the conventional contrasts for means separation. Drip loss 2 data includes contrasts that focuses on comparing non-moisture enhanced product vs. moisture enhanced product (Contrast 2) and to examine drip loss within non-moisture enhanced product (Contrast 3). For the loin, drip loss was greater ($P < 0.001$; Contrast 2) in ME vs SS steaks. The increase in drip loss with ME is most likely due to the general influx of the CaAsc solution volume into the meat. Capillary forces between the thick and thin myofilaments can hold water in the meat; however these long range electrostatic (capillary) forces only retain a pre-determined amount of solution (Hui et al., 2001). Since interfilament spacing controls how much water a myofibril can hold, excess moisture or solution will be expelled as drip loss (Lawrie and Ledward, 2006) as found in the present study. Aalhus et al. (2000) found that skeletal separation did not affect drip loss values versus control steaks when evaluating the *longissimus*. This was evident in the present study as drip loss 1 was similar ($P = 0.07$; Contrast 1) for the NPP steaks compared to SS steaks as well as all other PPT (Table 12). As interfilament spacing increases, it is possible that SS treatments employed more capillary forces releasing less moisture than steaks not treated with SS since interfilament spacing may be increased in size. While drip loss 1 was similar ($P = 0.07$) in BRIT vs CONT (Table 13), the presence of a PPT by breed interaction ($P < 0.01$) was most likely attributed to the large breed difference in drip loss for SS/ME product and all other PPT. Drip loss 1 values for BRIT sourced ME steaks were over 200% greater than BRIT sourced SS steaks but did not result in PPT by breed

interactions between NPP and all other PPT ($P = 0.06$; Contrast 1). BRIT sourced ME steaks had a more substantial ($P < 0.001$; Contrast 3) increase in drip loss than SS steaks with the converse true for CONT.

Drip loss 2 data examines drip loss differences in ME steaks versus NPP and SS where moisture enhancement was not used with the hypothesis that adding moisture in processing should increase drip loss. Drip loss 2 was greater ($P < 0.001$; Contrast q) in ME steaks vs. the average of NPP and SS steaks while drip loss was greater ($P < 0.01$; Contrast p) in NPP vs. SS steaks (Table 12). The use of SS increased retention of moisture in the product which is also the trend when combining SS and ME as there were no differences ($P > 0.41$; Contrast r) in drip loss between SS/ME steaks versus all other PPT. The PPT by breed interaction ($P < 0.001$; Contrast q) for Drip loss 2 data (Table 14) is due to the high drip loss for ME steaks from BRIT cows as previously described. In terms of consumer experience, the drip loss seen with all PPT is 2-3% which would be very hard, if possible, to distinguish the losses apart.

Lipid oxidation is often assessed in product after display by examining discolouration based on objective measures of colour and the amount of malondialdehyde (MDA) present in the meat using the TBARS assay. There were no differences ($P > 0.15$) in TBARS across PPT, suggesting MDA content was similar for ME product vs. all other PPT. Use of ascorbic acids in the past decreased lipid oxidation, prevented discolouration, and (or) creation of a warmed over flavour. (Craig et al., 1996; Sanchez-Escalante et al., 2001; Cheng and Ockerman, 2007). As stated previously, ascorbic acid can prevent metmyoglobin (MetMb) formation as well as lipid oxidation by acting as an oxygen molecule scavenger and act with natural tocopherols in meats to restrict

formation of fat peroxides and lipid and pigment oxidation (Renner, 1990; Ismail et al., 2009). Lawrence et al. (2003a) reported a decrease in lipid oxidation when using CaAsc on beef from steer and heifer carcasses; using the same 0.2M CaAsc solution as the present study. Dipping beef in a 10% CaAsc solution decreased lipid oxidation and delayed metmyoglobin formation (decolouration or browning) (Mitsumoto et al., 1991). However the MDA levels for all PPT in the present study are greater than the 0.10 mg/kg MDA threshold established by Cheng and Ockerman (2007) where discernable cardboardy, painty or unpalatable off-flavours may occur. If these findings hold true, the decrease in MDA for the moisture enhanced steaks is not drastic enough to suggest a potential inhibition of lipid oxidation by CaAsc injection alone. MDA content (Table 13) did not differ ($P > 0.44$) across breeds.

3.4.7 INITIAL DAY OF PROCESSING: RIBEYE

The overall analysis of variance for initial day of processing for the ribeye muscle is presented in Appendix Table 4. Measurements on the initial day of processing included pH and CIE* colour values with the latter used for calculating hue angle and chroma. Initial day processing pH was similar ($P = 0.35$; Contrast 1) for control (NPP) steaks vs. all other PPT. This agrees with data for the LOIN (Table 12) and findings by Lawrence et al (2003a) evaluating CaAsc for moisture enhancement of strip loin steaks from youthful carcasses. While skeletal separation in the past did not affect product pH for the first 24 h and up to 6 d after slaughter (Aalhus et al., 2000), pH was greater ($P < 0.03$; Contrast 2) for ME vs. SS steaks. However, these differences are of questionable

biological significance. There was no difference ($P > 0.76$) in pH across breeds (Table 13) while the PPT by breed interaction ($P < 0.04$; Contrast 2) was previously described in the discussion for initial pH data for the LOIN. The addition of weak acids via injection into *longissimus* muscle (CaAsc; Lawrence et al., 2003a) or the *Pectoralis profundus* (lactic acid; Berge et al., 2001) decreased pH 1 to 28 h post-mortem contrary to findings for the present study. Explanations for potential mechanisms for pH change were previously discussed.

Steaks subjected to ME were lighter ($P < 0.03$; Contrast 2) than SS steaks; the differences in lightness (L^*) are very small and most likely would not be detected by the human eye. There were no differences in lightness between control and Tendercut steaks (Ludwig et al., 1997), with the latter procedure a modification of the SS technique used in the present study. The paler appearance of ME steaks may be due to activation of the calpains from calcium injection, enhancing degradation of myofibrils within the meat (Aktas and Kaya, 2001). BRIT sourced steaks were lighter ($P < 0.02$) than CONT sourced steaks (Table 16). Hue angle was similar ($P > 0.25$) across PPT and between breeds. Chroma was greatest ($P < 0.001$; Contrast 1) in NPP steaks vs. all other PPT. Colour intensity or purity may become dull when steaks are injected with a ME solution. Since solutions may contain pro-oxidant properties (CaAsc) or humectant properties (salt based solutions), the additional solution retained may reflect light differently as exudation may occur if enhancement exceeds the physical space within the myofibers (Lawrie and Ledward, 2006). This may be responsible for lower ($P < 0.02$; Contrast 2) chroma in ME vs SS steaks and lower ($P < 0.01$; Contrast 3) in SS/ME vs. the average of SS and ME steaks. Use of skeletal separation procedures in the past produced rib eye steaks with

decreased redness, an increase in brown hue, and a duller colour intensity when compared to control steaks (Claus et al., 1997; Aalhus et al., 1999). Chroma values were similar ($P > 0.56$) across breeds (Table 16).

3.4.8 14 DAY AGED PRODUCT: RIBEYE

The pH values for SS/ME steaks were lower ($P < 0.05$; Contrast 1) than the average of SS and ME steaks (Table 15). The biological significance of this difference is questionable as the pH values fall within the range of pH for normal beef as previously described. Although ascorbic acid solution decreased pH in ground beef over 7 and 14 d of storage due to its inherent acidity (Gok et al., 2009), this was not evident in the present study. There were no breed differences ($P = 0.67$) in pH for 14 d aged ribeyes (Table 16). A PPT by breed interaction ($P < 0.01$; Contrast 2) exists with pH for SS steaks greater for CONT vs. BRIT while the converse was true for ME steaks (Table 17). The biological significance of this interaction is questionable. Lightness (L^*) values were lower ($P < 0.001$; Contrast 1) for NPP steaks vs. all other PPT while SS/ME steaks were lighter ($P = 0.03$; Contrast 3) than the average of both SS and ME steaks (Table 15). While there were no breed differences ($P > 0.62$; Table 17) in L^* , the presence of a PPT by breed interaction ($P < 0.04$; Contrast 1) is of questionable biological significance as the L^* values are within 5 units (Table 17) across all PPT and breeds. These differences in lightness are most likely indistinguishable to the human eye based on pork colour grading standards (NPPC, 2000). These findings are consistent with past studies using weak acids which lower pH. The ionic presence of the weak acids causes swelling of

muscle proteins creating a more spatially increased structure along with causing light reflection that scatters the light and makes the product appear lighter (Onenc et al., 2004; Serdaroglu et al., 2007). Hue angle was lower ($P < 0.001$; Contrast 1) in NPP steaks vs. all other PPT (Table 15). This concurs with past studies where ME with CaAsc increased hue angle (Lawrence et al., 2003a) in LOIN steaks while use of skeletal separation increased hue angle vs. control steaks (Aalhus et al., 1999). Hue angle was similar ($P > 0.93$) between breeds (Table 16). Chroma was greater ($P < 0.02$; Contrast 2) in SS vs ME steaks (Table 15). As was described previously, the scattering of light by swollen meat can decrease colour intensity or vividness. Chroma was similar ($P > 0.19$) across breeds (Table 16).

3.4.9 SHELF LIFE DETERMINATION: RIBEYE

After retail display, there were no differences ($P > 0.33$) in product pH across PPT (Table 15) or between breeds (Table 16). Lightness values were similar ($P > 0.41$; Contrast 1) between NPP steaks and all other PPT. There were no differences in L^* between control and Tendercut rib eye steaks in the past (Ludwig et al., 1997). Product lightness was highest ($P = 0.04$; Contrast 3) for SS/ME steaks vs. the average of SS and ME steaks. There were no breed differences ($P > 0.76$) in lightness (Table 16). Although hue angle was lower ($P < 0.04$; Contrast 2) in ME steaks compared to SS steaks (Table 15), there were no breed differences ($P > 0.39$) in hue. Chroma was greater ($P < 0.001$; Contrast 1) in NPP steaks vs. all other PPT agreeing with findings for 14 day aged product. ME steaks had a lower ($P < 0.001$; Contrast 2) chroma (colour intensity) than

SS steaks (Table 15); similar to shelf life findings for LOIN steaks (Table 12). The SS/ME steaks also had a lower ($P = 0.004$; Contrast 3) chroma than the average of SS and ME steaks. The present findings are in agreement with Aalhus et al. (2000), where skeletal separation did not affect chroma value vs. control steaks. The decrease in chroma suggests that ME caused colour washout (decrease in colour vividness) versus steaks not injected with CaAsc. There were no breed differences ($P > 0.05$) in chroma (Table 16).

Following shelf life evaluation, drip loss 1 was greater ($P < 0.001$; Contrast 2) in ME vs. SS steaks while drip loss was greater ($P < 0.03$; Contrast 3) in SS/ME steaks versus the average of SS and ME steaks. Aalhus et al. (2000) found that skeletal separation did not affect drip loss values vs. control steaks while Lawrence et al. (2003a) found higher drip loss with strip loins when injecting CaAsc solutions versus non-injected control steaks. While drip loss 1 was similar ($P > 0.68$) between breeds (Table 16), a PPT by breed interaction (Table 17) was present ($P < 0.03$; Contrast 3) as drip loss 1 was greater for CONT vs. BRIT with SS/ME steaks while the converse was true for ME and SS steaks. The 1-2% difference in drip loss would likely not be detected by a consumer. Drip loss 2 was greater ($P < 0.001$; Contrast q) in ME vs. SS and NPP steaks in agreement with postmortem processing treatment differences for LOIN (Table 15). Drip loss 2 was also higher in SS/ME vs. all other PPT ($P = 0.006$; Contrast r) concurring with Lawrence et al. (2003a) that injection of CaAsc can increase drip loss vs. non-injected *longissimus* steaks. However, this contrasts with findings for the LOIN in the present study along with similar drip loss value between NPP and SS steaks ($P > 0.15$; Contrast p). The PPT by breed interaction ($P < 0.03$; Contrast t) for Drip loss 2 data

(Table 17) is due to greater drip loss for SS/ME steaks from CONT vs. BRIT cows while the converse was true for all other PPT.

Based on TBARS values, lipid oxidation was greater ($P < 0.01$; Contrast 2) in SS vs. ME steaks (Table 15) and would suggest a potential pro-oxidant effect when the muscle is stretched. This difference cannot be fully explained at the present time while MDA levels in the present study were similar ($P > 0.45$; Contrast 1) for NPP steaks versus all other PPT (Table 15). Lipid oxidation was similar ($P > 0.75$) between breeds (Table 16). Although changes in the lipid oxidation may not be visibly evident through the formation of metmyoglobin, the presence of lipid oxidation over 0.60 mg MDA/kg can produce other detrimental effects (Campo et al., 2006) which will be discussed in a subsequent section.

3.4.10 INITIAL DAY OF PROCESSING: *SEMIMEMBRANOSUS*

The overall analysis of variance for initial day of processing for the *semimembranosus* muscle (SM) is presented in Appendix Table 5. Measurements on the initial day of processing included pH and CIE* colour values with the latter used for calculating hue angle and chroma. There were no differences ($P > 0.54$) in pH, L^* , or hue angle across PPT (Table 18) or between breeds (Table 19). Past work on SM (Aalhus et al., 1999) found similar hue values for skeletal separated and control sides with red and brown hues. Chroma was greater ($P < 0.02$; Contrast 1) in NPP vs. all other PPT most likely due to lower ($P < 0.01$; Contrast 2) values for ME versus SS. Lawrence et al. (2003a) found that colour deterioration increased vs. control sides when injecting SM

with CaAsc or CaCl₂ due to myoglobin oxidation with moisture enhancement. Colour deterioration progress can be associated with decreases in a* value, increase in hue angles, and a decrease in chroma (Hui et al., 2001). Chroma was similar ($P > 0.94$) between breeds (Table 19).

3.4.11 14 DAY AGED PRODUCT: *SEMIMEMBRANOSUS*

The pH values for 14 d aged product were similar ($P > 0.47$) across PPT (Table 18) and between breeds (Table 19). Lightness values were lower ($P < 0.02$; Contrast 2) in SS versus ME steaks. However, this difference is less than two L* units which, as previously described, surmounts to visually similar products according to standards set by NPPC (2000). Lightness values were also similar ($P > 0.57$) between breeds (Table 19). There were no differences ($P > 0.17$) in hue angle or chroma across PPT (Table 18) or between breeds (Table 19). The present findings are supported by past work using CaCl₂ on SM where moisture enhancement did not affect product appearance based on colour (Lansdell et al., 1995).

3.4.12 SHELF LIFE DETERMINATION: *SEMIMEMBRANOSUS*

While pH was lower ($P < 0.02$; Contrasts 1 and 2) between NPP and all other PPT (Contrast 1) and between SS and ME steaks (Contrast 2), the differences are of questionable biological significance as pH values across PPT were within the previously outlined “normal” ultimate pH levels found in beef. Skeletal separation did not affect pH

of *longissimus* or ST muscle from the round in the past (Aalhus et al., 2000). There were no breed differences ($P > 0.29$) in pH (Table 19). Lightness values were similar ($P > 0.71$) across all PPT and between breeds. While hue angle was similar ($P > 0.90$; Contrast 1) between NPP and all other PPT, hue angle was greater (more brown, less red) ($P < 0.01$; Contrast 2) for ME versus SS steaks. Similar to other muscles in this study, discolouration occurring from the pro-oxidant effects of ascorbic acid (Lawrie and Ledward, 2006) or potentially CaAsc (Lawrence et al., 2003a) have been documented to cause browning from metmyoglobin formation. This discolouration is not desirable as colour is very important for consumers who will often avoid packages of discoloured meat unless they are discounted. A more red coloured top loin steak or ground beef was preferred versus purchasing product that was more brown or purple coloured (Carpenter et al., 2001). Adding rosemary extracts or vitamin C decreased discolouration when used in tandem with calcium salts (Wheeler et al., 1996; Lawrence et al., 2004). Although no breed differences were found ($P > 0.46$; Table 19) for hue angle, a PPT by breed interaction was present ($P < 0.05$; Contrast 2) (Table 20) due to a much larger difference in hue between breeds for ME vs. SS steaks. Chroma values were lower ($P < 0.05$; Contrast 3) in SS/ME steaks vs. the average of the SS and ME steaks. As chroma values were similar when comparing NPP steaks versus all other PPT ($P > 0.55$; Contrast 1) and similar ($P > 0.25$; Contrast 2) when comparing SS vs. ME steaks, the chroma values suggest similar colour vividness or intensity between injected and non-injected steaks. Lower chroma values for SS/ME steaks suggest that more metmyoglobin oxidation may have occurred with the combination PPT. There were no breed differences ($P > 0.63$) for chroma (Table 19). The colour intensity differences (chroma) across all PPT are no more

than 3-4 units; consumers may not be able to detect these differences.

After retail display simulation, drip loss 1 was lower ($P < 0.02$; Contrast 1) between NPP steaks and all other PPT. This difference is most likely due to the effects of moisture enhancement as drip loss was lower ($P < 0.01$; Contrast 2) in SS vs. ME steaks. Aalhus et al. (2000) examined skeletal separation procedures for processing the ST from the round and found no differences in drip loss vs. control steaks. As ageing time increases, the electrostatic forces holding the CaAsc brine within the meat could decrease in strength due to the myofibril degradation which occurs through the process of ageing (Hui et al., 2001), with resultant increases in drip loss. There were no breed differences ($P > 0.17$) in drip loss (Table 19). For drip loss 2 data, drip loss was greater in SS/ME steaks ($P = 0.02$; Contrast r) vs. other PPT although the biological significance of the difference is questionable. Furthermore, drip loss 2 increased ($P < 0.03$) in ME vs. SS and NPP steaks (Contrast q; Table 18). There were no differences ($P = 0.925$; Contrast p) in drip loss for NPP vs. SS steaks.

In terms of lipid oxidation, MDA content was greater ($P < 0.03$; Contrast 2) in SS vs. ME steaks (Table 18) which is similar to the effect of SS on ribeye steaks (Table 15). Again, this effect cannot be explained at the present time as there were no differences ($P > 0.79$; Contrast 1) between NPP and all other PPT. MDA content was greater ($P < 0.01$) in BRIT vs. CONT steaks (Table 19), indicating increased lipid oxidation which could result in increased warmed-over flavour for BRIT steaks when evaluated by a trained sensory panel. Campo et al. (2006) found levels of 0.7 mg MDA/kg or higher correlated well with sensory evaluations resulting in some cases of abnormal and rancid beef flavour with lower overall acceptability in beef strip loin steaks. While the MDA content

may differ between PPT, the biological significance in terms of meat product is debatable. In this study, levels of MDA never exceeded 1 mg MDA/kg for any PPT while Campo et al. (2006) deduced a threshold of 2 mg MDA/kg before lipid oxidation was detected by all trained sensory evaluators in strip loin steaks. The small changes in MDA content after simulated display may occur naturally without influence from the administered PPT. MDA levels for untreated beef loins averaged 0.5-0.6 mg MDA/kg when vacuum sealed for 0 and 7 d post-mortem (Kim et al., 2002); MDA levels in the present study may not be associated with the presence of warmed over flavour.

3.4.13 INITIAL DAY OF PROCESSING: *SEMITENDINOSUS*

The overall analysis of variance for initial day of processing for the *semitendinosus* muscle is presented in Appendix Table 6. Measurements on the initial day of processing included pH and CIE* colour values with the latter used for calculating hue angle and chroma. Initial day pH for this muscle was similar ($P > 0.25$) across the PPT (Table 21) and breeds (Table 22). Lightness and hue angle values were similar ($P > 0.15$) across PPT (Table 21) and breeds (Table 22). Past studies found ST to be darker (Grobbel et al., 2008) and potentially more colour stable (Janz et al., 2005; Seyfert et al., 2006) than the *triceps brachii* and *longissimus* muscle when moisture enhanced with a lactic acid or phosphate salt based solution. Chroma values were greater ($P < 0.001$; Contrast 1) for NPP steaks vs. all other PPT. This may be attributed to the effects of moisture enhancement on chroma (scattering of light on the surface) as chroma was greater ($P < 0.001$; Contrast 2) in SS vs. ME steaks. In addition, chroma was lower ($P <$

0.04: Contrast 3) in SS/ME steaks vs. the average of SS and ME (Table 21). The ST steaks behaved similarly to SM steaks from moisture enhancement as colour intensity was lower in ME vs. SS steaks. Although hue angles were comparable across PPT suggesting no differences in discolouration, the ME treatment dulled colour intensity as compared to non-injected steaks. The injection of CaAsc into *longissimus* muscle decreased chroma value vs. non-injected control steaks or distilled water injected steaks (Lawrence et al., 2003a). Colour scores decreased while discolouration increased 40% when *longissimus* was moisture enhanced with as little as 0.15 M CaCl₂ (Diles et al., 1994). Chroma (colour intensity) did not differ ($P > 0.43$) between breeds (Table 22).

3.4.14 14 DAY AGED PRODUCT: SEMITENDINOSUS

The pH values for 14 d aged steaks were greater ($P = 0.05$; Contrast 1) for NPP vs all other PPT. This is most likely due to the effects of moisture enhancement as pH was lower ($P < 0.05$; Contrast 2) in ME vs. SS steaks. The biological significance of these differences is questionable. There was no difference ($P > 0.39$) in pH between breeds (Table 22). Lightness value were greater ($P = 0.001$; Contrast 2) for SS vs. ME steaks (Table 21) but it is doubtful the human eye will detect this difference. There was no difference ($P > 0.45$) in L* between breeds (Table 22). Hue angle was similar ($P > 0.16$) across PPT (Table 21) and breeds (Table 22) suggesting little to no differences in colour or presence of discolouration. NPP steaks had the highest ($P < 0.001$; contrast 1) chroma value (colour intensity) when compared to all other PPT. Chroma was lower ($P < 0.05$) for BRIT vs. CONT steaks suggesting a less vivid, more washed out lean colour in BRIT

steaks.

3.4.15 SHELF LIFE DETERMINATION: *SEMITENDINOSUS*

After retail simulation, pH did not vary ($P > 0.22$) across PPT (Table 21) and breeds (Table 22). ME steaks were lighter ($P < 0.001$; Contrast 2) than SS steaks (Table 21) while lightness did not vary ($P > 0.45$) between breeds (Table 21). NPP steaks had a lower hue angle ($P < 0.04$; Contrast 1) versus all other PPT. This is most likely due to the effects of moisture enhancement as hue angle was lower ($P < 0.01$; Contrast 2) for SS vs. ME steaks. Retail display or “shelf-life” of meat products were extended for muscles in the top and bottom round when injecting an aqueous solution of 0.5%-1% ascorbic acid into whole sub-primal meat cuts (Wheeler et al., 1996). Sanchez-Escalante et al. (2001) also injected ascorbic acid yielding an increase in a^* values (increased redness) in beef patties. The injection of the ascorbic acid was reported to aid in sequestering free radicals and to prevent discolouration. Contrary to this work, injecting 0.2 M CaAsc into the SM or ST muscles increased hue angle (more brown), creating a more off-coloured and dark appearance (Lawrence et al., 2003a). Lawrence et al. (2003a) found CaAsc injected steaks to be less red (lower a^*) and less yellow (lower b^*) than control steaks. In contrast to what Lawrence et al. (2003a) hypothesized, the present study found increased oxidation occurring with CaAsc injection compared to NPP or SS steaks in terms of lipid oxidation (TBARS). Lawrence et al. (2003a) suggested that with increasing molar concentrations of CaAsc injected into muscle, larger amounts of free electrons become available for catalyzing metmyoglobin formation. However, this was

not evident for moisture enhanced ST after retail display in the present study. There were no breed differences ($P > 0.72$) in hue angle (Table 22). Chroma value did not vary ($P > 0.15$) across PPT (Table 21) or breeds (Table 22) similar to findings for SM steaks previously discussed.

Drip loss 1 after retail display was lower ($P < 0.001$; Contrast 2) in SS vs. ME steaks. As was explained in section 3.4.6., the use of SS on steaks may increase interfilament spacing and capillary forces which will decrease drip loss (Lawrie and Ledward, 2006). While there were no breed differences ($P > 0.20$; Table 22) in drip loss, a PPT by breed interaction was present ($P < 0.03$; Contrast 3) due to large breed differences in drip loss for ME steaks vs. SS/ME steaks (Table 23). For drip loss 2 data, drip loss in ME steaks was greater ($P < 0.001$; Contrast q) than SS and NPP steaks (Table 21) further supporting the previous discussion regarding increased drip loss with moisture enhancement using CaAsc. However, use of both SS and ME processes resulted in similar ($P > 0.25$; Contrast r) drip loss versus all other PPT. Similar drip loss ($P = 0.59$; Contrast p) was found with NPP vs. the use of only SS. MDA content after retail display was similar ($P = 0.08$) across all PPT (Table 21), suggesting that SS and (or) ME did not increase lipid oxidation vs. controls. While MDA levels were greater ($P < 0.05$) in BRIT vs. CONT steaks (Table 22), this difference would not be easily detected by consumers. Use of ascorbic acid in the past decreased lipid oxidation in round roasts when injected to 5% by weight (Cheng and Ockerman, 2007). The ST tends to be more susceptible to lipid oxidation than SM (Xiong and Kupski, 1999) suggesting that moisture enhancement may not affect MDA levels. In the present study, moisture enhancement did not reduce MDA levels in the SM or ST.

3.4.16 AEROBIC PLATE COUNT

A shelf life study was conducted with 14 day aged steaks which were weighed, placed on styrofoam trays and overwrapped with PVC film. The packaged steaks were chilled for an additional 4 days with determination of aerobic plate counts to assess if PPT used in the present study influenced microbial growth which could compromise food safety. Aerobic plate counts were not affected ($P > 0.43$) by PPT for RE (with APC ranging from 11-17 cfu/cm²; Table 15) and ST (with APC ranging from 13-19 cu/cm²; Table 21). There were no breed differences ($P > 0.08$) in APC for RE (with APC ranging from 10-18 cfu/cm²; Table 16) or ST (with APC ranging from 11-16 cfu/cm²; Table 22). Aerobic plate counts were not affected ($P > 0.43$) by PPT for RE (with APC ranging from 11-17 cfu/cm²; Table 15) and ST (with APC ranging from 13-19 cu/cm²; Table 21). There were no breed differences ($P > 0.08$) in APC for RE (with APC ranging from 10-18 cfu/cm²; Table 16) or ST (with APC ranging from 11-16 cfu/cm²; Table 22). These results agree with previous work with CaAsc injection where APC from injected steaks did not differ from a non-injected control or distilled water injected steaks (Lawrence et al., 2003a). The injection of CaAsc into whole muscles in the present study did not decrease aerobic plate counts in fabricated steaks compared to NPP steaks, suggesting that the injection of this solution does not lower nor increase counts when injecting the whole muscle. Although CaAsc has minimal ability to limit bacterial growth (Mukherjee et al., 2008) when injected into whole beef muscles, the injection of other moisture enhancement solutions decreased or did not affect aerobic plate counts when various

acids including ascorbic acid (Anderson and Marshall, 1990) and CaAsc (Lawrence et al., 2003a; Mukherjee et al., 2008) were used.

The additional handling of the carcass with skeletal separation (SS, SS/ME) also did not increase microbial growth suggesting that this postmortem processing technique may be implemented without necessarily compromising food safety. This study concurs with Lawrence et al. (2003) that CaAsc cannot decrease nor increase aerobic bacterial counts consistently when injected into whole muscles.

3.4.17 SHEAR FORCE AND COOKLOSS

The overall analysis of variance (ANOVA) for shear force determinations is presented in Appendix Table 7. The NPP LOIN steaks had the highest shear force ($P < 0.001$) vs. all other LOIN PPT (Table 24; Contrast 1). While shear force averaged lower for ME and SS treatments as compared to NPP, there were no differences ($P > 0.39$; Contrast 2) in shear force between the two single method PPTs. The lowest ($P < 0.001$; Contrast 3) shear force was found using combination SS/ME treatment vs. the average of both SS and ME treatments. (Table 24) This result suggests an additive effect when combining both the SS and ME treatments to further decrease shear force rather than using a single method PPT. Shear force data are similar for LOIN and RE for all contrasts (Table 24). The decrease in WBSF with SS in the present trial is supported by past studies where SS techniques decreased WBSF and increased taste panel ratings for myofibrillar, connective tissue, and/or overall tenderness evaluation in beef from youthful carcasses (Wang et al., 1994; Ludwig et al., 1997; Soerheim and Hildrum, 2002) Claus

et al. (1997) further suggested that the Tendercut procedure for SS decreases shear force and also the variation in finished product tenderness. The stretching of the *longissimus* from severing the connective tissue and vertebrae around the ribeye and loin sections allowed for an increase in sarcomere length for the *longissimus* muscle (Aalhus et al., 2000). Muscle stretching due to skeletal separation decreased WBSF values for *Gluteus medius*, *Rectus femoris* and *longissimus* from USDA Choice (Wang et al., 1994; Claus et al., 1997) and USDA Select carcasses (Claus et al., 1997). Skeletal separation decreased WBSF in the posterior rib and anterior loin in cull cows (Mandell et al., 2006). However, skeletal separation procedures have increased sensory tenderness ratings without increasing sarcomere length (Beatty et al., 1999; Shanks et al., 2002a).

The use of ME in cull cow beef has decreased shear force using calcium chloride (Whipple and Koohmaraie, 1993), CaAsc (Lawrence et al., 2003a) or sodium or calcium based phosphates (Baublits et al., 2006a) as well as lactic acid (Berge et al., 2001) as the primary ingredients in the ME solution. Injection of a 10% CaCl₂ solution into mature cattle increased myofibrillar breakdown and decreased WBSF values by at least 30% during ageing (Baohua Kong et al., 2006). The mode of action for tenderness enhancement using Ca-based solutions is via activation of m-calpain. While WBSF has decreased and taste panel ratings for tenderness have increased in the past using these methods of PPT, this is the first study to our knowledge that has combined the 2 procedures (SS/ME). The resulting additive effect for decreasing shear force in cow beef shows promise for further improvements in tenderness over a single method PPT. This may be especially important for enhancing the tenderness of cull cow beef which can be inconsistent in tenderness due to high amounts of connective tissue.

For SM steaks, NPP had the highest shear force ($P < 0.001$; Contrast 1) vs. all other PPT (Table 24). The use of ME decreased shear force ($P < 0.001$; Contrast 2) vs. SS. The failure of SS to decrease WBSF for SM may be related to the inability of this PPT to increase sarcomere length in this muscle. Improvements in tenderness with SS procedures are generally associated with increases in sarcomere length. However, Beaty et al. (1999) found only limited increases in sarcomere length compared to past studies albeit with the procedure increasing tenderness ratings. The findings in the present study contrast to Mandell et al. (2006) where skeletal separation increased sarcomere length and decrease WBSF in cull cow SM steaks. While shear force for SS/ME is lower ($P < 0.02$) than the average shear force of SS and ME, WBSF values were similar between ME and SS/ME. Moisture enhancement in the past decreased shear force in SM vs. control steaks from steers or bison (Boles and Swan, 1997; Boles and Shand, 2001; Dhanda et al., 2002). Use of CaCl_2 for moisture enhancement of SM has also decreased shear force (Wheeler et al., 1991) and improved tenderness ratings (Lansdell et al., 1995).

WBSF values were not affected ($P > 0.20$; Contrast 1) by postmortem processing of the ST vs. NPP. While ME with (without) SS improved WBSF values for SM, this was not the case for ST. The failure of skeletal separation to decrease WBSF for the ST may be due to the procedure only creating tension on specific muscles (Wang et al., 1994; Claus et al., 1997; Ludwig et al., 1997) as sarcomere length was only increased in the LOIN and RE and not in muscles from the round (SM, ST) (Table 10). Beaty et al. (1999) used the Tendercut™ process on SM and ST muscles from youthful carcasses and found sarcomere length to increase in contrast to the present study but with minimal effects on myofibril fragmentation indices or WBSF. Large amounts of connective tissue

in the ST may also be responsible for the lack of improvement with SS and ME (Torrescano et al., 2003). The limited improvement in WBSF from ME is most likely related to differential response to moisture enhancement for certain muscles including muscles from the round. (DeYonge-Freeman et al., 2000)

Shear force values for the LOIN decreased ($P < 0.001$; Table 25) as postmortem ageing increased (Table 25) with the greatest ($P < 0.001$; Contrast 1) WBSF for 7 d aged steaks vs. the average WBSF for 14 and 21 d aged steaks. Shear force values were lower ($P < 0.03$; Contrast 2) for 21 vs. 14 d aged steaks. Postmortem ageing decreased shear force in beef up to 28 d postmortem (Hui et al., 2001) due to the actions of the calpain enzyme system resulting in myofibrillar breakdown (Lawrie and Ledward, 2006). Similar responses to postmortem ageing were found for RE steaks. Postmortem processing treatment by postmortem aging interactions ($P < 0.01$) for WBSF were present for the LOIN and RE when examining NPP vs all other PPT by 7 vs. 14 and 21 days of ageing (Table 26). As postmortem ageing continues after 7 days, there is a greater decrease in WBSF with NPP vs. all other PPT. Since product was not assessed under 7 days of ageing, it appears the largest decrease in WBSF occurred from death to 7 days of ageing for carcasses that underwent skeletal separation and (or) *longissimus* muscles that were moisture enhanced. Further ageing decreased WBSF but not to the extent found with NPP steaks. Seven day aged SS/ME steaks and 14 d aged ME and SS steaks were as tender as NPP steaks aged 21 days; the additive effect of combining SS and ME reduced the ageing time required for tenderization versus using a single PPT. This effect was observed by Ludwig et al. (1997) using the Tendercut technique with ageing creating an additive effect when combined with skeletal separation. Baohua Kong et al. (2006)

reported the use of CaCl_2 on the *longissimus* muscle had shortened the ageing time required to achieve adequate tenderness by at least 30%. A PPT by postmortem ageing interaction was present ($P < 0.03$; Contrast 2) for RE steaks aged for 14 vs. 21 d aged product. Again, the largest decrease in WBSF between 14 and 21 d of aging was for NPP vs. all other PPT. Similar results have been achieved using CaCl_2 injection into mature cow strip loins by decreasing 14 d aged WBSF values by 2.59 kg in non-injected strip loins to only 0.35 kg for injected strip loins when compared to WBSF values for 1 d aged control and injected steaks (Morgan et al., 1991). The use of the combination SS/ME treatment may replace the need for ageing the meat product past one week increasing product turnover and potentially increasing shelf life as meat would be sold quicker.

There was no benefit for ageing SM steaks past 7 days vs the average of 14 and 21 d ageing ($P > 0.42$; Contrast 1) nor for WBSF ($P = 0.06$; Contrast 2) for 21 vs. 14 d aged product (Table 25). In contrast, ageing ST steaks for at least 14 d decreased ($P < 0.001$; Contrast 1) WBSF vs. 7 d aged product. However there was no further improvement ($P > 0.93$; Contrast 2) with 21 d ageing. Postmortem ageing is well documented to decrease shear force in the *longissimus* over the course of 1-21 d postmortem with the majority of WBSF decrease occurring during the first 14 d postmortem (Alarconrojo and Dransfield, 1995; Aalhus et al., 2004; Bruce et al., 2004). Mandell et al. (2006) found a decrease in WBSF for the SM sourced from cull cows when steaks were aged for at least 14 d. In contrast, ageing for up to 21 d vs. 14 d did not decrease WBSF for SM sourced from youthful cattle (Aalhus et al., 2004). Aalhus et al. (2004) suggested that cut-specific minimum and maximum ageing times should be developed for industry to further improve product quality.

Cook losses for LOIN and RE were similar ($P \geq 0.11$; Contrast 1) between NPP and all other PPT (Table 24). This is most likely attributed to a mean decrease in cook losses for SS steaks while cook losses tended to increase using moisture enhancement (ME, SS/ME). This is supported by greater ($P < 0.03$; Contrast 3) cook losses for SS/ME vs. the average of SS and ME. Hoffman et al. (2006) found no differences in cook loss between cow beef (*longissimus* muscle) moisture enhanced with sodium phosphates and lactate vs. non-injected steaks. Lawrence et al. (2003) similarly did not find any differences in cooking loss with any Ca based ME treatments including CaAsc, CaCl_2 , and calcium lactate solutions. Work using CaCl_2 injection on *longissimus* and round muscles increased cooking losses for muscles from the round (Wheeler et al., 1991) and strip loins from mature cows (Diles et al., 1994). Past work evaluating skeletal separation found no differences in cooking losses versus control steaks (Claus et al., 1997; Ludwig et al., 1997). The range in cook loss in the present study for LOIN and RE steaks (18.6-22.2%) is similar to cook losses for mature beef broiled to 70 °C injected with CaCl_2 and aged up to 14 d (Morgan et al., 1991; Diles et al., 1994). The findings in the present study are supported by Wang et al. (1994) who found no differences in thawing loss, cook loss, and moisture content between non-skeletally separated steaks and skeletally separated steaks using the Tendercut procedure. Cook losses in SM and ST steaks were similar ($P = 0.06$; Contrast 1) for NPP vs. all other PPT. There were no differences in cook loss between SS and ME steaks ($P > 0.34$; Contrast 2) nor was there an additive effect with SS/ME ($P > 0.10$; Contrast 3). Use of phosphates and lactate on cow beef SM increased cook losses in the past vs. non-injected steaks (Hoffman, 2006).

These authors attributed this increase to a compact myofibrillar structure in the SM resulting in less water being bound within the physical structure of the muscle.

Postmortem aging effects on cook loss were similar for all muscles with the greatest cook losses ($P < 0.01$; Contrast 1) with 7 d aged product vs. lower losses in 14 and 21 d aged product (Table 25). Ageing for 21 d did not affect ($P > 0.24$; Contrast 2) cook losses for muscles from the round (SM, ST). While cook losses were greater ($P < 0.001$; Contrast 2) in 21 vs. 14 d aged product for the LOIN, the differences are of questionable biological significance. Cooking losses were similar when whole *supraspinatus* and *triceps brachii* muscles were injected with a moisture enhancing salt-phosphate solution (Lennon et al., 2006). Findings from the present study agree with past studies where postmortem processing treatment did not affect cook losses; this suggests that neither injection of calcium into the whole muscles or SS treatments increased cooking losses across the various postmortem ageing times.

3.4.18 SENSORY EVALUATION OF THE RIBEYE

The ANOVA tables for the sensory evaluation trials are presented in Appendix tables 8 (Rib eye) and 9 (*Semitendinosus*). Sensory evaluation ratings for all 4 “tenderness” attributes (softness, tenderness, chewiness, rate of breakdown) were lower ($P < 0.001$; Contrast 1) for NPP steaks vs. all other PPT (Table 27). This is in agreement with the present study’s WBSF data examining the effects of postmortem processing treatments (Table 24). In support of the present findings, a trained taste panel found strip loin steaks moisture enhanced with a 0.2M CaAsc solution to be softer and more tender

than non-treated, control steaks (Lawrence et al., 2003a). These authors used USDA select grade beef (Canadian AA) from youthful carcasses to evaluate the benefits of moisture enhancement with CaAsc on myofibrillar tenderness, overall tenderness, juiciness and flavour. All calcium ascorbate injected steaks yielded a higher overall tenderness and myofibrillar tenderness than that of non-injected control steaks. Similar effects were found when injecting strip loins with calcium chloride (Wheeler et al., 1997b) or sodium phosphate solutions (Vote et al., 2000) with lower shear force values and higher taste panel ratings for softness and tenderness. Studies involving the *longissimus* muscle that has been treated with a skeletal separation technique found increased myofibrillar tenderness (Ludwig et al., 1997) for USDA Select beef as well as overall tenderness ratings (Ludwig et al., 1997; Aalhus et al., 1999; Aalhus et al., 2000) for USDA Select or Choice beef compared to control steaks. Ludwig et al. (1997) did not find an increase in any tenderness (myofibrillar or overall) rating in Tendercut steaks vs. control steaks even though sarcomere length was drastically increased with skeletal separation for all RE steaks.

There were no differences ($P > 0.13$; Contrast 2) in ratings for 3 of the 4 “tenderness” attributes between SS and ME with the exception that chewiness ratings were greater ($P < 0.03$) for ME vs. SS product. The injection of CaAsc increased the perceived energy required to masticate the product vs. skeletally separated product. Steaks that were SS may have had increased inter-filament spaces with reoriented stretched fibers creating a perception of less energy to chew through it. Taste panellists rated CaAsc injected strip loins as having more connective tissue than control steaks suggesting a chewier piece of meat (Lawrence et al., 2003a). However, Lawrence et al.

(2003a) also found differences in connective tissue among all moisture enhancement treatments (non-enhanced, control or moisture enhancement with CaAsc, calcium lactate, and calcium chloride) with control steaks having the most connective tissue. In contrast to what was found, these authors suggested that if cooking procedures as well as muscle was kept constant, then the differences in chewiness and connective tissue may likely be an artefact of variation in myofibrillar tenderness rather than actual differences in connective tissue quality or quantity. All 4 “tenderness” attributes were rated highest ($P < 0.02$; Contrast 3) for SS/ME steaks compared to SS and ME steaks. There appears to be an additive effect from combining a skeletal separation procedure with moisture enhancement to tenderize beef similar to WBSF data (Table 24). Shear force values for 14 d ribeye SS/ME steaks averaged 3.77 kg. Rodas-González et al. (2009) reported an 81% satisfaction rate amongst trained panellist ratings when shear force values were ≤ 4.09 kg. This suggests a potential for 81% consumer satisfaction in terms of tenderness for SS/ME steaks provided shear force methodologies are similar between the 2 studies. There were no differences ($P > 0.46$; Contrast 9) in tenderness attributes between the 2 breeds or PPT by breed interactions ($P > 0.24$; Contrasts 6, 7, 8) for tenderness attributes. The absence of breed differences in this study agree with previous literature where no breed differences in *longissimus* muscle tenderness was found in French cull cows (Dranfield et al, 2003). Similarly, muscle fiber tenderness and overall tenderness were rated alike between BRIT and CONT *longissimus* muscle steaks (Schnell et al., 1997)

The hypothesis that combining the 2 PPT may produce an additive effect on enhancing tenderness vs. use of a single method PPT lead to development of an a priori contrast. This contrast compares SS/ME vs. Red Branded beef with the latter including

AA (USDA Select equivalence) and AAA (USDA Choice equivalence) marbled beef that is currently marketed at Canadian grocery stores. Softness and tenderness attributes were similar ($P > 0.14$; Contrast 4) between SS/ME and Red Branded product. However, panellists found SS/ME steaks chewier ($P < 0.06$; Contrast 4) with a slower rate of breakdown ($P < 0.04$; Contrast 4) than red branded beef with these attributes suggesting less breakdown of connective tissue and myofibrillar proteins in the cull cow beef. There were no differences ($P > 0.42$; Contrast 5) in tenderness attributes between AA and AAA marbled beef. Although fat composition is more abundant with AAA beef, tenderness perception would seem to be a product comprised of more than just the presence of intramuscular fat.

Initial and overall juiciness ratings were lower ($P \leq 0.02$; Contrast 1) in NPP vs. all other PPT. The increase is due to moisture enhancement with CaAsc as ratings were lower ($P < 0.01$; Contrast 2) in SS vs. ME product while there were no differences ($P > 0.63$; Contrast 3) between SS/ME and the average of SS and ME. Moisture enhancement via injection with CaAsc (Lawrence et al., 2003a), CaCl_2 , weak organic acids (Aktas and Kaya, 2001) and salt phosphates (Lennon et al., 2006) have increased tenderness and juiciness vs. untreated steaks. Juiciness ratings in *longissimus* muscles were similar between SS and control steaks (Ludwig et al., 1997; Aalhus et al., 1999) when evaluated by trained panellists. However, a following study completed by Aalhus et al. (2000) showed that mean consumer scores for juiciness (hedonic scale based and descriptive) of the *longissimus* muscle were higher for SS treated steaks than controls. A PPT by breed interaction ($P < 0.01$; Contrast 8) was present for initial and overall juiciness ($P = 0.01$; Contrast 8) with lower ratings for BRIT vs. CONT with SS/ME steaks while the converse

was true for SS and ME steaks. In contrast, Schnell et al. (1997) found no differences in juiciness ratings between BRIT and CONT cull cows fed a high concentrate diet. Red branded beef products were rated lower ($P < 0.01$; Contrast 4) for initial and overall juiciness than SS/ME. The injection of CaAsc and using skeletal separation together increased juiciness perception by the influx of CaAsc solution that was retained within the myofibers. While initial juiciness was greater ($P < 0.05$; Contrast 5) in AAA vs. AA marbled beef, there were no differences ($P > 0.12$; Contrast 5) in overall juiciness across marbling grades of Red Branded beef.

Flavour ratings were similar ($P > 0.61$; Contrasts 1, 2, 3) across PPT for cull cow beef with no differences between NPP and all other PPT (Contrast 1), SS vs. ME (Contrast 2), and SS/ME vs. the average of SS and ME (Contrast 3). Aalhus et al. (1999, 2000) also found that skeletal separation did not affect flavour (desirability and intensity) vs. control steaks. In terms of moisture enhancement, Lawrence et al. (2003) found contradictory evidence to the present study, suggesting that injection of CaAsc decreased beef flavour intensity; use of 0.1 M concentration injection solutions produced the most beef flavour while the least beef flavour was found with steaks moisture enhanced with 0.3 M solutions. The SS/ME steaks were rated having more intense ($P < 0.04$; Contrast 4) beef flavour than Red Branded beef. Flavour ratings for AAA marbled beef exceeded ($P < 0.03$; Contrast 5) ratings for AA marbled product (Table 28); this is most likely due to marbling grade differences as higher intramuscular fat in AAA marbled beef can impart a stronger flavour than meat with less fat content (Dransfield, 2008). In contrast to flavour ratings, off-flavour ratings were lower ($P < 0.03$; Contrast 1) in NPP vs. all other PPT. This is most likely due to moisture enhancement as off-

flavour ratings were greater ($P < 0.04$; Contrasts 2 and 3) in ME vs SS (Contrast 2), and SS/ME vs. the average of SS and ME (Contrast 3). Panellists characterized off-flavours as rancid, gamey, sour or metallic for the study with sour, salty, and metallic used to describe off-flavours in ME and SS/ME beef from moisture enhancement using CaAsc. Lawrence et al. (2003a) reported problems with off-flavours and colour deterioration when CaAsc was used in solution at concentrations greater than 0.1 M. Use of CaCl_2 for moisture enhancement also resulted in undesirable off-flavour characteristics including bitter, metallic, and livery (Morgan et al., 1991), as well as sour and oniony flavours. (Morris et al., 1997). Lipid oxidation may have also contributed to the off-flavour intensity and may be responsible for similar ($P > 0.15$; Contrast 4) off-flavour ratings between SS/ME and Red Branded beef. Off-flavours including cardboard-like and warmed over flavours may be detected from an MDA equivalent presence of only 0.10 mg MDA/ kg (Cheng and Ockerman, 2007). However, Campo et al. (2006) suggested that at least 0.6-2.0 mg MDA/ kg must be present before consumers start to detect rancidity or decreases in beef flavour. In the present study, the off-flavour is likely being imparted by the CaAsc solution injected into the steaks as described previously.

3.4.19 SENSORY EVALUATION OF THE *SEMITENDINOSUS*

Sensory evaluation ratings from evaluation of the *semitendinosus* are presented in Table 28. NPP steaks were rated the lowest ($P \leq 0.05$; Contrast 1) in softness and chewiness compared to all other PPT with no differences ($P > 0.08$; Contrast 1) in tenderness and rate of breakdown. Lawrence et al. (2003b) examined the effects of

moisture enhancement on sensory evaluation of the *semitendinosus*; there were no differences amongst ME with calcium chloride, ME with calcium phosphate, and non-injected controls for any taste panel attribute (myofibrillar tenderness, juiciness, beef flavour intensity, connective tissue, and off-flavour). Their results suggested that staged injection (injection of two solutions at different times) with CaCl_2 and calcium phosphate produced similar tenderness ratings as non-injected, control steaks. The findings for chewiness in the present study disagree with panellist ratings collected by Lawrence et al. (2003b) where ST steaks did not vary in chewiness or connective tissue across any calcium phosphate treatment. ST steaks from 30 month old cattle were injected with a phosphate/chloride solution; this ME treatment decreased WBSF compared to control steaks (Pietrasik et al., 2010). There were no differences in WBSF value for ST muscles from crossbred steers when various skeletal separation procedures on the hip were compared to control steaks (Shanks et al., 2002). In this study, there were no differences in ST tenderness attributes (softness, tenderness, chewiness or rate of breakdown) ($P > 0.37$; Contrast 2) between SS and ME steaks (Table 28). Mandell et al. (2006) investigated the effects of SS (MOLAS) on the WBSF of ST muscles sourced from cull cows and found no differences versus steaks from untreated, control carcass sides. SS/ME steaks had similar tenderness attributes ($P > 0.68$; Contrast 3) as ME and SS steaks suggesting no benefit in tenderization when PPT are combined as compared to the single method PPT. Decreases in WBSF are less important when sensory panellists can not detect any improvements in tenderness attributes (Hostetler et al., 1973).

Softness, tenderness, chewiness and rate of breakdown were rated lower ($P < 0.001$; Contrast 4) overall for the combination SS/ME than ratings for AA and AAA red

branded beef product (Table 28) which contrasts with findings for softness and tenderness evaluation of ribeye (Table 27). According to panellists, the use of SS and(or) ME did in fact create a softer and less chewy product; however, the improvement was not adequate enough to guarantee comparable ratings in tenderness for cull cow beef in comparison to the red branded, AA/AAA beef steaks. Work completed by Huffman et al. (1996) suggested that 51% of consumers ranked tenderness as the most important trait for retail purchased meat products while 39% chose flavour, and 10% chose juiciness. A follow up to this study showed that consumers were willing to pay higher prices for more tender products and could distinguish different levels of tenderness in a product (Boleman et al., 1997). Sensory panellist ratings for untreated ST steaks from USDA Select/Choice steers were lower for tenderness and beef flavour intensity than ratings for *longissimus* muscle (Carmack et al., 1995). Generally, muscles from the loin are more tender, soft, and juicy than muscles from the round (Carmack et al., 1995) which suggests that postmortem treatment may be required if ST are to be marketed as a tender product. The tenderness ratings for cull cow beef correlate well with the shear force values discussed previously; the ME steaks had the highest rated tenderness out of all PPT while SS/ME steaks had the lowest WBSF. On the contrary, the SS steaks had the highest WBSF values, while NPP steaks had the lowest panellist tenderness ratings. The SS steaks were rated similar ($P > 0.37$; Contrast 2) to ME steaks for softness, tenderness, juiciness, and flavour; there were also no differences ($P > 0.68$; Contrast 3) in ratings for the 4 “tenderness” attributes between SS/ME and the average of SS and ME. Beaty et al. (1999) found similar WBSF and myofibrillar breakdown values for Tendercut and control steaks from cooked ST muscles. There were no differences ($P > 0.05$; Contrast 5)

in any palatability attribute between AA and AAA red branded product. Steaks sourced from BRIT and CONT carcass sides were rated similarly ($P > 0.06$; Contrast 9) for softness, tenderness, chewiness, and rate of breakdown. Previous work evaluating breeds for carcass characteristics and eating quality found similar rating for myofibrillar tenderness, chewiness, and instrumental shear force for ST steaks from British and Continental cull cows (Schnell et al, 1997). These results agree with our study suggesting that breeds were rated similarly for tenderness-based palatability traits. There were no PPT by breed interactions ($P > 0.23$; Contrasts 6, 7, and 8) for any “tenderness” attribute.

Previously, injecting calcium phosphate or lactate into the ST did not improve product juiciness or flavour (Lawrence et al., 2003b). Use of SS and (or) ME did not affect ($P > 0.11$; Contrast 1) initial juiciness vs. NPP but overall juiciness was lower ($P = 0.05$; Contrast 1) in NPP steaks vs. all other PPT. While there were no differences ($P > 0.05$; Contrasts 2 and 3) in initial and overall juiciness between SS and ME steaks (Contrast 1) and SS/ME vs. the average of SS and ME (Contrast 2), initial and overall juiciness ratings were greater ($P < 0.02$; Contrast 4) for combination SS/ME steaks vs. AA/AAA red branded beef steaks. This suggests that the SS/ME treatment increased initial juiciness beyond ratings for high quality Canadian beef from youthful cattle (USDA Select/Choice equivalence) that has not been moisture enhanced. Hoffman et al. (2008) used sodium phosphate, potassium phosphate and lactate blends for moisture enhancement to increase overall juiciness of the ST muscle vs. control steaks. Juiciness was rated higher for steaks injected with sodium phosphates and/or sodium chloride as compared to non-moisture enhanced steaks (Vote et al., 2000; Pietrasik et al., 2010).

There were no PPT by breed interactions ($P > 0.12$; Contrasts 6, 7, and 8) for initial or overall juiciness.

Use of SS and (or) ME did not affect ($P > 0.16$; Contrast 1) beef flavour in NPP steaks vs. all other PPT. Beef flavour was rated more intense ($P < 0.05$; Contrast 2) for SS vs. ME steaks. Additionally, beef flavour was more intense ($P < 0.02$; Contrast 4) for SS/ME cull cow beef steaks vs. red branded, AA/AAA beef steaks. Lawrence et al. (2003) injected strip loin muscles with CaAsc yielding similar beef flavour intensities for control and moisture enhanced steaks. Beef flavour intensity was greater ($P < 0.03$; Contrast 9) in BRIT vs. CONT steaks. This finding disagrees with a previous study where beef flavour for cull cow beef was similar between BRIT and CONT breeds (Schnell et al., 1997). However, a PPT by breed interaction ($P < 0.03$; Contrast 7) for beef flavour intensity was due to large differences in flavour intensity between breeds for SS vs. ME steaks.

Off-flavour ratings were lower ($P = 0.001$; Contrast 1) in NPP steaks vs. all other PPT. There appears to be an additive effect on off flavour by combining SS with ME as off-flavour ratings were greater ($P < 0.05$; Contrasts 3 and 4) for SS/ME steaks vs. the average of SS and ME (Contrast 3), and for SS/ME vs. red branded AA/AAA steaks (Contrast 4). Potentially, stretching myofibers may increase exposed surfaces to lipid oxidation creating an off-flavour. Moisture enhancement has produced metallic, bitter, salty, or sour off-flavours when injected or marinated into specific beef muscles using CaAsc (Lawrence et al., 2003b), commercial salt mixtures (Hoffman, 2006), or lactic acid (Berge et al., 2001). Moisture enhancement with the 0.2 M CaAsc in the present study created an off-flavour described as metallic, bitter, oily, soapy, or livery by

individual panellists. Lawrence et al. (2003) reported similar descriptions of off-flavours when ST steaks were moisture enhanced with brine concentrations greater than 0.1 M using CaAsc, calcium lactate, or calcium chloride. The use of 0.2 M CaAsc in the present study was based on the findings of Lawrence et al. (2003a) where this intermediate concentration of brine enhanced tenderness vs. using 0.1 M CaAsc but produced an off flavour intensity rating between traces and practically none. ME did create a noticeable off-flavour in ME treated steaks in this study, similar to that Lawrence et al. (2003a) found using an injection of 0.2 M CaAsc.

3.5 CONCLUSIONS AND IMPLICATIONS

The aim of this study was to investigate the effects of skeletal separation (MOLAS) and moisture enhancement separately as single methods of postmortem processing and for potential additive effects when combining the processes for improving the eating quality of cull cow beef. Both objective measures of overall meat quality and sensory evaluation of tenderness, juiciness, and flavour were used to evaluate the effectiveness of the postmortem processing procedures. Ultimately, the study is designed to evaluate potential processing procedures for the beef industry to enhance the eating quality of cull cow beef in smaller scale packing plants where SS could be incorporated into an existing station. Larger packers may see scope with this study and adapt it for application in large scale operations as incorporation of another station would be required.

Carcass characteristics were not affected when using postmortem processing techniques. Differences in backfat and *longissimus* muscle area were present between the

British and Continental cull cows which is expected when comparing diverse frame types of cattle. The skeletal separation procedure successfully increased carcass length by approximately 17-18 cm; the increase in carcass length was similar at both 1 and 24 h postmortem while not differing across breeds. The increase in carcass length was due to stretching of the muscles as evidenced by increases in sarcomere length for the ribeye and strip loin sections of the *longissimus*. The skeletal separation procedure did not affect sarcomere length for muscles from the round (*semimembranosus*, *semitendinosus*) requiring a modified or different SS technique to successfully increase sarcomere length.

In general, pH was similar for the *longissimus* (ribeye and strip loin) and muscles from the round (*semimembranosus*, *semitendinosus*) across all postmortem processing treatments and for all durations of aging suggesting that the processing treatments did not actively change the ultimate pH values. Objective measures of lean colour were evaluated due to the importance of product colour for the consumer. While there were differences in L* amongst postmortem processing techniques, the differences were not great enough to be detected by the human eye. Hue angle, an indicator of discolouration was, however, highest in moisture enhanced product including 14 d aged RE and 21 d aged SM and ST. Moisture enhanced steaks had a tendency to be discoloured, being less attractively red and more brownish in colour. Colour intensity (chroma) often was lowest for ME steaks, suggesting the enhanced steaks may become more dull in colour due to injection with calcium ascorbate. A limitation of the current study was the failure to evaluate colour parameters on cooked product to determine the effects of the CaAsc on steak appearance immediately before consumers would typically eat the steaks. This may or may not reveal whether moisture enhancement affects the appearance of the product

before consumption.

The moisture enhanced steaks increased drip loss when compared to non-moisture enhanced steaks, with ME consistently having higher amounts of drip loss than NPP and SS steaks across all muscles examined. The level of MDA content decreased for those steaks injected with CaAsc; however, MDA levels did not decrease below the 0.10 mg/Kg MDA level at which Cheng and Ockerman (2007) determined there would be no discernable off-flavours. There were no differences amongst any postmortem processing treatments in APC counts. Thus the additional handling of the carcass from skeletal separation and (or) moisture enhancement did not promote or inhibit aerobic bacterial growth. Individually, skeletal separation effectively decreased shear force for the RE and LOIN while moisture enhancement using CaAsc decreased shear force for all muscles analyzed. The combination treatment (SS/ME) decreased shear force to a greater extent than using skeletal separation or moisture enhancement alone, suggesting an additive effect for enhancing postmortem tenderization. Postmortem aging of steaks for 7, 14, or 21 d was generally effective for decreasing shear force for the LOIN, RE and ST; however 21 d of aging was needed to further decrease shear force value for SM steaks as compared to 7 d aged product. There was a large decrease in shear force for 7 d aged RE and LOIN steaks with the combination SS/ME treatment compared to the control, NPP. Furthermore, the decrease in shear force found for aging the product from 7 to 21 d resulted in a moderate decrease in shear force for NPP steaks with little improvement in shear force for SS/ME steaks. The combination SS/ME treatment may be just as or more effective for enhancing postmortem tenderization than post-mortem aging.

Sensory evaluation of RE steaks revealed that combination SS/ME processing of

cull cow beef increased tenderness levels to values found with red branded AA/AAA steaks from youthful carcasses. Juiciness ratings for cull cow beef were equal or superior to ratings for red branded beef. While flavour intensity for cull cow beef was rated similarly to AA/AAA marbled red branded beef, off-flavours were detected in cull cow beef moisture enhanced with CaAsc. Sensory evaluation of the ST found that regardless of the postmortem processing treatment used, the cull cow beef was lower in tenderness than the red branded, AA/AAA steaks. Both juiciness and flavour ratings were higher for the cull cow beef versus the red branded beef. However, similar to sensory evaluation of the RE, off-flavours were detected in cull cow beef moisture enhanced with CaAsc.

Ultimately, the use of skeletal separation and moisture enhancement via CaAsc solution injection was beneficial for increasing sarcomere length, decreasing shear force, increasing tenderness and creating a juicier and more flavourful product. However, off-flavours may be present when using CaAsc for moisture enhancement. Further studies using consumer panels would be beneficial to determine if the off-flavours are a problem when consumers typically prepare beef at home using seasonings, sauces, and condiments. The integration of both skeletal separation and moisture enhancement using CaAsc may help create value-added products from cull cow beef which may increase returns for cow-calf producers. A value-added steak could be marketed as a premium product and may create a new market niche with similar quality to that of existing red branded products. Consumers can have a more consistent, higher quality product while producers can increase marketability of cull cow beef product.

Table 1. Carcass Side Combinations for Postmortem Processing Treatment ^z Allocation		
Treatment Combination	Treatment Allocation to Specific Carcass Side	
	Left Side of Carcass	Right Side of Carcass
1	NPP	SS
2	NPP	ME
3	NPP	SS/ME
4	SS	NPP
5	SS	ME
6	SS	SS/ME
7	ME	NPP
8	ME	SS
9	ME	SS/ME
10	SS/ME	NPP
11	SS/ME	SS
12	SS/ME	ME

^z Treatments= No Postmortem Processing (NPP), Skeletal Separation (SS), Moisture Enhancement (ME), or Skeletal Separation and Moisture Enhancement (SS/ME).

Table 2. Steak Allocation for Meat Quality Evaluation of Cull Cow Beef

		Steak Number							
Muscle	1	2	3	4	5	6	7	8	9
<i>Longissimus</i> muscle from the ribeye ²	14 day aged product: Taste Panel Evaluation	14 day aged product: Determination of Shear force	21 day aged product: Determination of Shear force	7 day aged product: Determination of Shear force	14 day aged product: Determination of TBARS	14 day aged product: Determination of Colour, pH,	14 day aged product: spare	1 day aged product: Determination of Colour and pH, Sarcomere length	n/a*
<i>Longissimus</i> muscle from the loin ³	1 day aged product: Determination of Colour and pH, Sarcomere length	14 day aged product: Determination of Colour, pH	14 day aged product: Determination of TBARS, Driploss	14 day aged product: Determination of Shear force	21 day aged product: Determination of Shear force	7 day aged product: Determination of Shear force	n/a	n/a	n/a
<i>Semimembranosus</i> ^{x,u,t}	Origin ^a 7 day aged product: Determination of Shear force	14 day aged product: Determination of Shear force	21 day aged product: aged Determination of Shear force	14 day aged product: Determination of TBARS, Driploss	14 day aged product: Determination of Colour, pH	7 day aged product: Determination of Shear force	14 day aged product: Determination of Shear force	21 day aged product: aged Determination of Shear force	Insertion ¹ 1 day aged product: Determination of Colour, pH, Sarcomere Length
<i>Semitendinosus</i> ^{w,s,t}	Origin ^s 14 day aged product: Determination of TBARS, Driploss	14 day aged product: Determination of Colour, pH	7 day aged product: Determination of Shear force	21 day aged product: Determination of Shear force	14 day aged product: Taste Panel Evaluation	14 day aged product: Determination of Shear force	14 day aged product: Taste Panel Evaluation	n/a	Insertion ¹ 1 day aged product: Determination of Colour, pH, Sarcomere Length
² - Ribeye: The muscle was fabricated into steaks starting at the posterior end of the rib at the 12 th /13 th rib interface with Steak 1 being the first steak fabricated, while Steak 8 was located at the anterior end in the chuck.									
³ - Loin: The muscle was fabricated into steaks starting at the anterior end of the loin at the 12 th /13 th rib interface with Steak 1 being the first steak fabricated, while Steak 7 was located at the posterior end of the loin near the sirloin.									
^x - <i>Semimembranosus</i> : The muscle was fabricated into steaks starting near the adductor muscle with Steak 1 being the first steak fabricated, while Steak 8 was located near the <i>Semitendinosus</i> . Steak 9 was also cut near the adductor.									
^w - <i>Semitendinosus</i> : The muscle was fabricated into steaks starting at the ischial end of the muscle with Steak 1 being the first steak fabricated, while Steak 7 was also cut at the ischial end of the muscle.									
^u - <i>Semimembranosus</i> origin represents muscle interaction with the ventral surface of the ischial tuberosity.									
^t - <i>Semimembranosus</i> insertion represents muscle interaction with the medial surfaces of the distal femur behind the collateral ligament.									
^s - <i>Semitendinosus</i> origin represents muscle interaction with the ischial tuberosity.									
^r - <i>Semitendinosus</i> insertion represents muscle interaction with the medial surface of tibia.									
* - n/a is indicated where the steak was either not used or created									

Table 3. Contrast Coefficients Used for Means Separation Across Postmortem Processing Treatments				
Contrast	Postmortem Processing Treatment (PPT) Coefficients			
	No Postmortem Processing	Skeletal Separation	Moisture Enhancement	Combination Skeletal Separation and Moisture Enhancement
1	3	-1	-1	-1
2	0	1	-1	0
3	0	-1	-1	2
Contrast 1 = Comparison of No Postmortem Processing (NPP) vs. All other Postmortem Processing Treatments.				
Contrast 2 = Comparison of Skeletal Separation vs. Moisture Enhancement using Ca Ascorbate.				
Contrast 3 = Comparison of combined Skeletal Separation and Moisture Enhancement using Ca ascorbate vs. the average of Skeletal Separation and Moisture Enhancement using Ca Ascorbate.				

Table 4. Contrast Coefficients Used for Means Separation with Postmortem Processing Treatments by Breed Interactions														
Contrast Coefficients to Evaluate Postmortem Processing Treatments by Breed Interactions Involving Carcass Characteristics, Carcass Drop, and Meat Quality Attributes														
		Postmortem Processing Treatments ² (PPT) for Cull Cow Beef												
		No Postmortem Processing (NPP)			Skeletal Separation (SS)			Moisture Enhancement (ME)			Combination Skeletal Separation and Moisture Enhancement (SS/ME)			
Contrast	Description of Individual Contrast	BRIT ^y	CONT		BRIT	CONT		BRIT	CONT		BRIT	CONT		
1	Interaction of NPP vs. all other PPT by breed	3	-3		-1	1		-1	1		-1	1		1
2	Interaction of SS vs. ME by breed	0	0		-1	1		1	-1		0	0		0
3	Interaction of SS/ME vs. the average of SS and ME by breed	0	0		-1	1		-1	1		2	-2		-2
Contrasts Coefficients to Evaluate Postmortem Processing Treatments by Breed Interactions Involving Sensory Evaluation of Rib Eye and <i>Semitenidosus</i> Steaks														
		Postmortem Processing Treatments (PPT) for Cull Cow Beef												
		Red Branded Beef ^z			NPP			SS			ME			
Contrast	Description of Individual Contrast	AA	AAA		BRIT	CONT		BRIT	CONT		BRIT	CONT		
1	Interaction of NPP vs. all other PPT	0	0		3	3		-1	-1		-1	-1		-1
2	SS vs. ME	0	0		0	0		-1	-1		1	1		0
3	SS/ME vs. the average of SS and ME	0	0		0	0		-1	-1		-1	-1		2
4	AA and AAA Red Branded Products vs. ME/SS	1	1		0	0		0	0		0	0		-1
5	Red Branded AA vs. AAA Product	1	-1		0	0		0	0		0	0		0
6	Interaction of NPP vs. all other PPT by breed	0	0		3	-3		-1	1		-1	1		1
7	Interaction of SS vs. ME by breed	0	0		0	0		-1	1		1	-1		0
8	Interaction of SS/ME vs. the average of SS and ME by breed	0	0		0	0		-1	1		1	1		-2
9	British vs. Continental	0	0		-1	1		-1	1		-1	1		1
^z Postmortem Processing Treatments for cull cow beef include NPP (no Postmortem Processing treatment), ME (Moisture Enhancement), SS (Skeletal Separation) and SS/ME (Combination Skeletal Separation and Moisture Enhancement).														
^y Breed represents either predominantly British (BRIT) or predominantly Continental (CONT) animals.														
^x Red branded beef includes AA/AAA graded RE and ST steaks sourced from steers and heifers.														

Table 5. Contrast Coefficients Used for Means Separation of Postmortem Ageing (Days)			
Contrast	Day of Postmortem Ageing		
	7	14	21
1	2	-1	-1
2	0	1	-1
Contrast 1 = Comparison of 7 versus the average of 14 and 21 days postmortem ageing.			
Contrast 2 = Comparison of 14 versus 21 days of postmortem ageing .			

	Postmortem Processing Treatments (PPT)														
	No Postmortem Processing (NPP)				Skeletal Separation				Moisture Enhancement				Combination Skeletal Separation and Moisture Enhancement		
Days Ageing Contrast	7	14	21		7	14	21		7	14	21		7	14	21
1	6	-3	-3		-2	1	1		-2	1	1		-2	1	1
2	0	3	-3		0	-1	1		0	-1	1		0	-1	1
3	0	0	0		-2	1	1		2	-1	-1		0	0	0
4	0	0	0		0	-1	1		0	1	-1		0	0	0
5	0	0	0		-2	1	1		-2	1	1		4	-2	2
6	0	0	0		0	-1	1		0	-1	1		0	-2	2

Trait	Covariates ^z	Postmortem Processing Treatments (PPT)						Contrasts				
		No Postmortem Processing (NPP)	Skeletal Separation (SS)	Moisture Enhancement (ME)	SS/ME	Min	Max	SE	P>F	Contrast 1: NPP vs. all other PPT	Contrast 2: SS vs. ME	Contrast 3: SS/ME vs. average of SS and ME
Number of carcass sides used ^y	-	32	31	31	30	30	32	-	-	-	-	-
% British/Continental	-	46.9/53.1	38.7/61.3	25.8/74.2	30.0/70.0	25.8/53.1	46.9/74.2	-	-	-	-	-
Age at Slaughter, (yrs)	NA	6.1	6.1	5.9	5.5	3.2	11.9	0.46	0.571	0.541	0.678	0.257
Hot Carcass Wt., (kg)	A	422.7	412.7	434.7	410.5	300	559	9.77	0.115	0.754	0.121	0.289
Side Carcass Wt., (kg)	A	211.6	206.7	216.7	205.3	150	280	4.92	0.129	0.714	0.300	0.153
Backfat at ¼ position ^x , (mm)	A	18.0	17.7	18.3	16.8	0	40	1.55	0.920	0.813	0.766	0.542
Backfat at ½ position ^w	A	12.8	14.6	14.3	14.3	0	35	1.42	0.797	0.329	0.881	0.920
Backfat at ¾ position ^v	A	11.1	11.6	12.0	10.8	0	27	1.22	0.921	0.778	0.823	0.542
Grade Fat ^u , (mm)	A	10.8	10.8	13.0	10.1	0	27	1.31	0.881	0.745	0.241	0.274
Marbling Score ^t	NA	2.41	2.23	2.28	2.06	0	4	0.16	0.423	0.209	0.821	0.337
LMA ^s , cm ²	A	96.3	99.0	98.1	97.0	64.53	138.59	2.37	0.819	0.510	0.785	0.605

^z Covariates; Age at Slaughter (A) and Grade Fat (G) were included where appropriate in the models and were noted here when significant at P < 0.10. Traits are labeled NA when the P > F value for all covariates is greater than 0.10.

^y Each cow was used for two postmortem processing treatments based on carcass side allocation.

^x Backfat at ¼ position = mm of Backfat in the first quadrant over the LM.

^w Backfat at ½ position = mm of Backfat in the second quadrant over the LM.

^v Backfat at ¾ position = mm of Backfat in the third quadrant over the LM.

^u Grade fat = mm of Backfat in the last quadrant over the LM.

^t Marbling Score – (0 = Devoid; 1 =Grade A; 2 =Grade AA; 3 =Grade AAA; 4=Prime).

^s Longissimus Muscle Area.

Table 8. Breed Effects on Carcass Characteristics of Cull Cow Beef

Trait	Covariates ³	Breed ²					P>F
		British	Continental	Min	Max	SE	
Number of carcass sides used ^x	-	44	80	44	80	-	-
Age at Slaughter, (yrs)	NA	6.0	5.8	3.2	11.9	0.27	0.590
Hot Carcass Wt, (kg)	A	427.1	413.2	300	559	6.80	0.344
Side Carcass Wt, (kg)	A	213.4	206.7	150	280	3.43	0.348
Backfat at ¼ position ^w , (mm)	A	18.9	16.5	0	40	1.08	0.127
Backfat at ½ position ^y	A	16.0	12.0	0	35	0.99	0.006
Backfat at ¾ position ^z	A	13.4	9.7	0	27	0.97	0.010
Grade Fat ¹ , (mm)	A	12.7	10.0	0	27	0.85	0.031
Marbling Score ⁵	NA	2.50	1.99	0	4	0.11	0.045
LMA ⁶ , cm ²	A	91.7	103.6	64.5	138.6	1.65	<0.001

² Breed designates cattle with predominantly British or Continental breeding.³ Covariates, Age at Slaughter (A) and Grade Fat (G) were included where appropriate in the models and were noted here when significant at P<0.10. Traits are labeled NA when the P>F value for all covariates is greater than 0.10.^x Each cow was used for two postmortem processing treatments based on carcass side allocation.^w Backfat at ¼ position = mm of grade fat in the first quadrant over the LM.^y Backfat at ½ position = mm of grade fat in the second quadrant over the LM.^z Backfat at ¾ position = mm of grade fat in the third quadrant over the LM.¹ Grade fat = mm of grade fat in the last quadrant over the LM.⁵ Marbling Score - (0 = Devoid; 1 =Grade A; 2 =Grade AA; 3 =Grade AAA; 4=Prime).⁶ Longissimus Muscle Area.

Table 9. Postmortem Processing Treatment by Breed Interaction on Carcass drop^z and Carcass Characteristics of Cull Cow Beef

Trait	Covariates ^v	Postmortem Processing Treatments ¹ by Breed ^x										Contrasts ^w		
		BRIT NPP	CONT NPP	BRIT SS	CONT SS	BRIT ME	CONT ME	BRIT SS/ME	CONT SS/ME	SE	P>F	Contrast 1	Contrast 2	Contrast 3
Number of carcass sides used ^u	-	15	17	12	19	8	23	9	21	-	-	-	-	-
Hot Carcass Wt., (kg)	A	427.5	417.8	412.7	412.8	461.8	407.6	406.4	414.6	13.96	0.093	0.172	0.038	0.377
Carcass Drop 24 h Postmortem Length (cm)	NA	-1.0	1.0	18.0	15.5	-2.8	0.5	17.8	16.5	1.42	0.209	0.505	0.049	0.543
Side Carcass Wt., (kg)	A	214.2	209.0	206.7	206.6	229.7	204.0	203.2	207.4	6.85	0.112	0.170	0.047	0.408

^zMeasurements of Pre- and Post-Skeletal Separation occurred within 1 h of slaughter. Carcass Drop measurements were taken at indicated time. Statistical model includes the following: Date of slaughter; Side = left or right carcass side; Side weight in kg; Age at slaughter in years; Grade fat = mm of grade fat in the last quadrant over the LM; Postmortem Processing Treatment which is described below; Breed= British or Continental; Postmortem Processing Treatment by Breed Interaction.

¹ Treatment= Either No Postmortem Processing (NPP), Skeletal Separation (SS), Moisture Enhancement (ME), or Skeletal Separation and Moisture Enhancement (SS/ME).

^x Breed represents either predominantly British (BRIT) or predominantly Continental (CONT) animals.

^w Contrast 1 = No Postmortem Processing (NPP) vs. All other Postmortem Processing Treatments by Breed ; Contrast 2 = SS vs. ME by Breed ; Contrast 3 =Combination (SS/ME) versus the average of SS and ME by Breed.

^v Covariates: Age at Slaughter (A), Date of Slaughter (D) and Grade Fat (G) were included where appropriate in the models and were noted here when significant at P<0.10. Traits are labeled NA when the P>F value for all covariates is greater than 0.10.

^u Each cow was used for two postmortem processing treatments based on carcass side allocation.

	Covariates ³	Postmortem Processing Treatments (PPT) ⁴				SE	P>F	Contrasts		
		NPP	SS	ME	SS/ME			Contrast 1: NPP vs. all other PPT	Contrast 2: SS vs. ME	Contrast 3: SS/ME vs. average of SS and ME
Pre-Skeletal Separation Carcass Length, cm	A, G	284.7	286.3	286.8	287.3	1.07	0.128	0.043	0.329	0.734
Post-Skeletal Separation Carcass Length, cm	G	286.8	303.8	287.3	304.0	1.30	<0.001	<0.001	<0.001	<0.001
Carcass Drop 1 h Postmortem, cm	A	1.0	18.0	0.0	18.0	0.89	<0.001	<0.001	<0.001	<0.001
Carcass Drop 24 h Postmortem, cm	A	0.5	17.0	-0.8	17.3	0.97	<0.001	<0.001	<0.001	<0.001
Sarcomere Length - Loin, μm	NA	1.95	2.28	1.95	2.31	0.052	<0.001	<0.001	<0.001	0.004
Sarcomere Length - Ribeye, μm	NA	2.05	2.52	2.05	2.41	0.082	<0.001	<0.001	<0.001	0.093
Sarcomere Length - <i>Semimembranosus</i> , μm	NA	1.94	1.93	1.95	1.95	0.026	0.892	0.892	0.538	0.683
Sarcomere Length - <i>Semitendinosus</i> , μm	NA	2.33	2.46	2.32	2.50	0.067	0.152	0.176	0.159	0.195

² -Measurements of Pre- and Post-Skeletal Separation occurred within 1 h of slaughter. Carcass Drop measurements were taken at indicated time.

³ -Treatment= Either No Postmortem Processing (NPP), Skeletal Separation (SS), Moisture Enhancement (ME) or Skeletal Separation and Moisture Enhancement (SS/ME).

⁴ Covariates; Age at Slaughter (A) and Grade Fat (G) were included where appropriate in the models and were noted here when significant at P<0.10. Traits are labeled NA when the P>F value for all covariates is greater than 0.10.

Table 11. Effects of Breed on Carcass Drop and Sarcomere Length Measurements ²				
	Breed ³		SE	P > F
	BRIT	CONT		
Pre-Skeletal Separation Carcass Length (cm)	285.2	285.5	0.99	0.944
Post-Skeletal Separation Carcass Length (cm)	294.6	294.4	1.01	0.860
Carcass Drop 1 h Postmortem (cm)	9.4	8.9	0.66	0.744
Carcass Drop 24 h Postmortem (cm)	8.2	8.4	0.76	0.888
Sarcomere Length - Loin, µm	2.12	2.12	0.046	0.948
Sarcomere Length - Ribeye, µm	2.25	2.27	0.047	0.745
Sarcomere Length - <i>Semimembranosus</i> , µm	1.95	1.95	0.022	0.995
Sarcomere Length - <i>Semitendinosus</i> , µm	2.42	2.39	0.050	0.668
² Measurements of Pre- and Post-Skeletal Separation occurred within 1 h of slaughter. Carcass Drop measurements were taken at indicated time.				
³ Breed represents either predominantly British (BRIT) or predominantly Continental (CONT) animals.				

Trait	Covariate ^x	Postmortem Processing Treatments ³ (PPT)					Contrasts			
		NPP	SS	ME	SS/ME	SE	P>F	Contrast 1: NPP vs. all other PPT	Contrast 2: SS vs. ME	Contrast 3: SS/ME vs. average of SS and ME
Initial Day of Processing										
% Solution Retained	A	-	-	9.0	11.6	0.31	<0.001	-	-	-
pH	NA	5.50	5.49	5.51	5.48	0.020	0.572	0.787	0.404	0.226
L*	A, G	32.6	32.4	31.8	31.8	0.43	0.176	0.106	0.171	0.491
Hue ^w	NA	9.4	10.0	8.7	10.7	0.68	0.102	0.499	0.085	0.059
Chroma ^v	G	20.8	20.7	19.4	20.0	0.30	<0.001	0.007	<0.001	0.935
14 Day Aged Product										
pH	NA	5.54	5.55	5.51	5.53	0.017	0.030	0.306	0.005	0.595
L*	A, G	32.8	31.7	33.0	33.9	0.45	0.001	0.839	0.022	0.002
Hue	G	11.4	10.8	12.6	12.6	0.69	0.078	0.360	0.044	0.221
Chroma	NA	21.1	21.5	20.7	20.5	0.27	0.014	0.349	0.018	0.062
Shelf Life										
pH	A	5.56	5.60	5.59	5.58	0.018	0.074	0.031	0.573	0.225
L*	A, G	32.2	31.7	32.6	32.7	0.39	0.076	0.742	0.054	0.129
Hue	A, G	13.5	13.5	14.1	14.6	0.58	0.314	0.294	0.355	0.228
Chroma	A, G	22.6	22.5	20.1	20.4	0.37	<0.001	<0.001	<0.001	0.037
Drip loss 1 ^s , %	NA	2.01	1.51	2.98	2.29	0.185	<0.001	0.069	<0.001	0.773
Drip loss 2, %	NA	2.01	1.51	2.98	2.29	0.185	<0.001	0.413 ^t	<0.001 ^q	0.003 ^p
TBARS ^u , mg MDA/kg ^l	NA	0.80	0.85	0.68	0.85	0.008	0.469	0.874	0.151	0.388
² Initial Day of Processing refers to samples taken after Moisture Enhancement where appropriate (approximately 24 h postmortem for all muscles); 14 Day Aged Product refers to steaks that have been aged at < 4°C until 14 d postmortem; Shelf Life refers to measurements on 18 d aged steaks where 14 d aged steaks are placed on foam trays and overwrapped with PVC film and stored at < 4°C in a chill cooler for an additional 4 d before sampling.										
³ Treatment= Either No Postmortem Processing (NPP), Skeletal Separation (SS), Moisture Enhancement (ME), or Skeletal Separation and Moisture Enhancement (SS/ME).										
^x Covariates: Age at Slaughter (A) and Grade Fat (G) were included in the model and noted here when significant at P<0.10. Traits are labeled NA when the P>F value for all covariates is greater than 0.10.										
^w Hue = Tan ⁻¹ (b*/a*); ^v Chroma = Square root of ((a*) ² + (b*) ²); ^u TBARS = Thiobarbituric Acid Reactive Substances; ^t MDA = Malonaldehyde.										
^q Drip loss 1 follows the conventional contrasts listed above; Drip loss 2 includes the contrasts: ^s SS/ME vs. All other PPT; ^p ME vs. SS and NPP; ^r ME vs. SS vs. NPP.										

Table 13. Effect of Breed on Traits Involving the <i>Longissimus</i> (Loin) on Initial day of Processing, After 14 days of Ageing, and After Evaluation of Shelf Life ^z					
		Breed ^y			
Trait	Covariate ^x	British	Continental	SE	P>F
Initial Day of Processing					
% Solution Retained	A	10.7	9.9	0.38	0.173
pH	NA	5.50	5.49	0.025	0.627
L*	A, G	32.5	31.8	0.44	0.277
Hue ^w	NA	9.0	10.4	0.72	0.187
Chroma ^v	G	19.9	20.6	0.29	0.110
14 Day Aged Product					
pH	NA	5.53	5.53	0.020	0.816
L*	A, G	32.8	32.9	0.44	0.873
Hue	G	11.8	11.9	0.68	0.990
Chroma	NA	20.7	21.2	0.25	0.143
Shelf Life					
pH	A	5.59	5.58	0.020	0.700
L*	A, G	32.5	32.1	0.42	0.512
Hue	A, G	14.2	13.6	0.57	0.472
Chroma	A, G	21.2	21.6	0.31	0.384
Drip loss, %	NA	2.48	1.91	0.211	0.066
TBARS ^u , mg MDA/kg ^t	NA	0.75	0.84	0.073	0.438
^z Initial Day of Processing refers to samples taken after Moisture Enhancement where appropriate (approximately 24 h postmortem for all muscles); 14 Day Aged Product refers to steaks that have been aged at < 4°C until 14 d postmortem; Shelf Life refers to measurements on 18 d aged steaks where 14 d aged steaks are placed on foam trays and overwrapped with PVC film and stored at < 4°C in a chill cooler for an additional 4 d before sampling.					
^y Breed designates cattle with predominantly British or Continental breeding.					
^x Covariates; Age at Slaughter (A) and Grade Fat (G) were included in the model and were noted here when significant at P<0.10. Traits are labeled NA when the P>F value for all covariates is greater than 0.10.					
^w Hue = $\tan^{-1} (b^*/a^*)$; ^v Chroma = Square root of $((a^*)^2 + (b^*)^2)$; ^u TBARS = Thiobarbituric Acid Reactive Substances; ^t MDA = Malonaldehyde.					

Table 14. Postmortem Processing Treatment by Breed Interactions for Traits Involving the <i>Longissimus</i> (Loin) on Initial day of Processing, After 14 days of Ageing, and After Evaluation of Shelf Life ^z														
Trait	Covariate ^y	Postmortem Processing Treatments ³ By Breed ^x								Contrasts ^w				
		BRIT	CONT	BRIT	CONT	BRIT	CONT	BRIT	CONT	SE	P>F	1	2	3
Initial Day of Processing														
pH	NA	5.50	5.50	5.48	5.51	5.54	5.48	5.49	5.47	0.028	0.102	0.409	0.021	0.811
Hue ^u	NA	9.1	9.7	8.8	11.3	7.1	10.3	11.0	10.3	0.58	0.054	0.354	0.614	0.010
14 Day Aged Product														
Hue	G	10.9	12.0	10.0	11.7	14.3	10.8	12.3	12.9	0.84	0.016	0.243	0.003	0.317
Chroma ^t	NA	20.5	21.8	21.1	21.9	21.0	20.3	20.1	20.9	0.38	0.041	0.065	0.037	0.253
Shelf Life														
Chroma	A, G	21.8	23.4	22.7	22.2	20.0	20.3	20.3	20.4	0.52	0.122	0.026	0.445	0.898
Drip loss 1 ^s , %	NA	2.10	1.92	1.60	1.41	3.72	2.25	2.52	2.07	0.260	0.003	0.057	0.001	0.244
Drip loss 2, %	NA	2.10	1.92	1.60	1.41	3.72	2.25	2.52	2.07	0.260	0.003	0.594 ^t	<0.001 ^q	0.982 ^p

^z Initial Day of Processing refers to samples taken after Moisture Enhancement where appropriate (approximately 24 h postmortem for all muscles); 14 Day Aged Product refers to steaks that have been aged at < 4°C until 14 d postmortem; Shelf Life refers to measurements on 18 d aged steaks where 14 d aged steaks are placed on foam trays and overwrapped with PVC film and stored at < 4°C in a chill cooler for an additional 4 d before sampling.

^y Treatment= Either No Postmortem Processing (NPP), Skeletal Separation (SS), Moisture Enhancement (ME), or Skeletal Separation and Moisture Enhancement (SS/ME).

^x Breed represents either predominantly British (BRIT) or predominantly Continental (CONT) animals.

^w Contrast 1 = No Postmortem Processing (NPP) vs. All other Postmortem Processing Treatments by Breed; Contrast 2 = SS vs. ME by Breed; Contrast 3 =Combination (SS/ME) versus the average of SS and ME by Breed.

^y Covariates; Age at Slaughter (A) and Grade Fat (G) were included in the model and were noted here when significant at P<0.10. Traits are labeled NA when the P>F value for all covariates is greater than 0.10.

^u Hue = Tan⁻¹ (b*/a*).

^t Chroma = Square root of ((a*)² + (b*)²).

^p Drip loss 1 follows the conventional contrasts listed above: Drip loss 2 includes the contrasts: ^sSS/ME vs. All other PPT; ^q ME vs. SS and NPP; ^rSS vs. NPP.

^z Initial Day of Processing refers to samples taken after Moisture Enhancement where appropriate (approximately 24 h postmortem for all muscles); 14 Day Aged Product refers to steaks that have been aged at < 4°C until 14 d postmortem; Shelf Life refers to measurements on 18 d aged steaks where 14 d aged steaks are placed on foam trays and overwrapped with PVC film and stored at < 4°C in a chill cooler for an additional 4 d before sampling.

^y Treatment= Either No Postmortem Processing (NPP), Skeletal Separation (SS), Moisture Enhancement (ME), or Skeletal Separation and Moisture Enhancement (SS/ME).

^x Breed represents either predominantly British (BRIT) or predominantly Continental (CONT) animals.

^w Contrast 1 = No Postmortem Processing (NPP) vs. All other Postmortem Processing Treatments by Breed; Contrast 2 = SS vs. ME by Breed; Contrast 3 = Combination (SS/ME) versus the average of SS and ME by Breed.

^v Covariates: Age at Slaughter (A) and Grade Fat (G) were included in the model and were noted here when significant at P<0.10. Traits are labeled NA when the P>F value for all covariates is greater than 0.10.

^u Hue = $\tan^{-1}(b^*/a^*)$.

^t Chroma = Square root of $((a^*)^2 + (b^*)^2)$.

^q Drip loss 1 follows the conventional contrasts listed above; Drip loss 2 includes the contrasts: ¹SS/ME vs. All other PPT; ²ME vs. SS and NPP; ³SS vs. NPP.

Table 15. Effects of Postmortem Processing Treatments on Traits Involving the <i>Longissimus</i> (Ribeye) on Initial day of Processing, After 14 days of Ageing, and After Evaluation of Shelf Life ^z										
Trait	Covariate ^x	Postmortem Processing Treatments ^y (PPT)					Contrasts			
		NPP	SS	ME	SS/ME	SE	P>F	Contrast 1: NPP vs. all other PPT	Contrast 2: SS vs. ME	Contrast 3: SS/ME vs. average of SS and ME
Initial Day of Processing										
% Solution Retained	A, G	-	-	12.4	13.2	0.31	0.054	-	-	-
pH	S	5.48	5.45	5.48	5.47	0.011	0.082	0.350	0.023	0.672
L*	G	34.0	33.4	34.6	33.2	0.41	0.057	0.590	0.023	0.089
Hue ^w	G	13.7	12.7	13.1	12.1	0.68	0.257	0.092	0.682	0.288
Chroma ^v	G	23.5	23.0	21.6	20.8	0.45	<0.001	<0.001	0.015	0.005
14 Day Aged Product										
pH	NA	5.55	5.55	5.53	5.51	0.015	0.050	0.104	0.308	0.047
L*	G	32.6	33.6	34.3	34.9	0.39	<0.001	<0.001	0.120	0.024
Hue	G	11.3	12.8	14.3	14.8	0.61	<0.001	<0.001	0.055	0.071
Chroma	G	21.7	22.1	21.0	21.4	0.50	0.106	0.561	0.018	0.777
Shelf Life										
pH	A	5.62	5.61	5.63	5.63	0.020	0.487	0.898	0.282	0.344
L*	A, G	33.1	33.2	33.1	33.9	0.39	0.190	0.412	0.794	0.042
Hue	A, G	14.5	15.0	13.7	14.9	0.50	0.173	0.945	0.037	0.306
Chroma	A, G	23.5	23.4	18.7	19.7	0.35	<0.001	<0.001	<0.001	0.004
Drip loss ¹ %, %	G	2.14	1.86	2.74	2.75	0.212	<0.001	0.055	<0.001	0.021
Drip loss 2, %	G	2.14	1.86	2.74	2.75	0.212	<0.001	0.006 ^t	<0.001 ^q	0.153 ^p
APC, cfu/cm ²	NA	11	15	17	12	3.0	0.189	0.151	0.157	0.576
APC, Log cfu/cm ²	NA	0.87	0.96	0.95	1.02	0.081	0.430	0.136	0.844	0.473
TBARS ^u , mg MDA/kg ^t	A	0.73	0.99	0.69	0.68	0.008	0.005	0.450	0.005	0.080
^z Initial Day of Processing refers to samples taken after Moisture Enhancement where appropriate (approximately 24 h postmortem for all muscles); 14 Day Aged Product refers to steaks that have been aged at < 4°C until 14 d postmortem; Shelf Life refers to measurements on 18 d aged steaks where 14 d aged steaks are placed on foam trays and overwrapped with PVC film and stored at < 4°C in a chill cooler for an additional 4 d before sampling.										
^y Treatment= Either No Postmortem Processing (NPP), Skeletal Separation (SS), Moisture Enhancement (ME), or Skeletal Separation and Moisture Enhancement (SS/ME).										
^x Covariates: Age at Slaughter (A) and Grade Fat (G) were included in the model and noted here when significant at P<0.10. Traits are labeled NA when the P>F value for all covariates is greater than 0.10.										
^w Hue = Tan ⁻¹ (b*/a*); ^v Chroma = Square root of ((a*) ² + (b*) ²); ^u TBARS = Thiobarbituric Acid Reactive Substances; ^t MDA = Malonaldehyde.										
^t Drip loss 1 follows the conventional contrasts listed above. Drip loss 2 includes the contrasts: ^q SS/ME vs. All other PPT; ^q ME vs. SS and NPP; ^p SS vs. NPP.										

^z Initial Day of Processing refers to samples taken after Moisture Enhancement where appropriate (approximately 24 h postmortem for all muscles); 14 Day Aged Product refers to steaks that have been aged at < 4°C until 14 d postmortem; Shelf Life refers to measurements on 18 d aged steaks where 14 d aged steaks are placed on foam trays and overwrapped with PVC film and stored at < 4°C in a chill cooler for an additional 4 d before sampling.

^y Treatment= Either No Postmortem Processing (NPP), Skeletal Separation (SS), Moisture Enhancement (ME), or Skeletal Separation and Moisture Enhancement (SS/ME).

^x Covariates: Age at Slaughter (A) and Grade Fat (G) were included in the model and noted here when significant at P<0.10. Traits are labeled NA when the P>F value for all covariates is greater than 0.10.

^w Hue = $\tan^{-1}(b^*/a^*)$; ^v Chroma = Square root of $((a^*)^2 + (b^*)^2)$; ^u TBARS = Thiobarbituric Acid Reactive Substances; ^t MDA = Malonaldehyde.

^q Drip loss 1 follows the conventional contrasts listed above; Drip loss 2 includes the contrasts: ^qSS/ME vs. All other PPT; ^qME vs. SS and NPP; ^qSS vs. NPP.

Table 16. Effect of Breed on Traits Involving the <i>Longissimus</i> (Ribeye) on Initial day of Processing, After 14 days of Ageing, and After Evaluation of Shelf Life ^z					
		Breed ^y			
Trait	Covariate ^x	British	Continental	SE	P>F
Initial Day of Processing					
% Solution Retained	A	13.2	12.5	0.37	0.249
pH	NA	5.47	5.47	0.010	0.765
L*	A, G	34.5	33.1	0.36	0.015
Hue ^w	NA	12.7	13.1	0.62	0.647
Chroma ^v	G	22.1	22.4	0.41	0.562
14 Day Aged Product					
pH	NA	5.54	5.53	0.016	0.667
L*	A, G	34.0	33.7	0.38	0.624
Hue	G	13.3	13.3	0.58	0.932
Chroma	NA	21.3	21.9	0.30	0.196
Shelf Life					
pH	A	5.64	5.61	0.023	0.332
L*	A, G	33.3	33.4	0.41	0.769
Hue	A, G	14.3	14.9	0.49	0.393
Chroma	A, G	21.0	21.7	0.25	0.057
Drip loss, %	NA	2.44	2.30	0.250	0.684
APC, cfu/cm ²	G	18	10	3.2	0.108
APC, Log cfu/cm ²	G	1.02	0.88	0.082	0.261
TBARS ^u , mg MDA/kg ^t	NA	0.76	0.78	0.06	0.753

^z Initial Day of Processing refers to samples taken after Moisture Enhancement where appropriate (approximately 24 h postmortem for all muscles); 14 Day Aged Product refers to steaks that have been aged at < 4°C until 14 d postmortem; Shelf Life refers to measurements on 18 d aged steaks where 14 d aged steaks are placed on foam trays and overwrapped with PVC film and stored at < 4°C in a chill cooler for an additional 4 d before sampling.

^y Breed designates cattle with predominantly British or Continental breeding.

^x Covariates; Age at Slaughter (A) and Grade Fat (G) were included in the model and were noted here when significant at P<0.10. Traits are labeled NA when the P>F value for all covariates is greater than 0.10.

^w Hue = Tan⁻¹ (b*/a*); ^v Chroma = Square root of ((a*²) + (b*²)).

^u TBARS = Thiobarbituric Acid Reactive Substances; ^t MDA = Malonaldehyde

^z Initial Day of Processing refers to samples taken after Moisture Enhancement where appropriate (approximately 24 h postmortem for all muscles); 14 Day Aged Product refers to steaks that have been aged at < 4°C until 14 d postmortem; Shelf Life refers to measurements on 18 d aged steaks where 14 d aged steaks are placed on foam trays and overwrapped with PVC film and stored at < 4°C in a chill cooler for an additional 4 d before sampling.

^y Breed designates cattle with predominantly British or Continental breeding.

^x Covariates: Age at Slaughter (A) and Grade Fat (G) were included in the model and were noted here when significant at P<0.10. Traits are labeled NA when the P>F value for all covariates is greater than 0.10.

^w Hue = $\tan^{-1}(b^*/a^*)$; ^v Chroma = Square root of $((a^*)^2 + (b^*)^2)$.

^u TBARS = Thiobarbituric Acid Reactive Substances; ^t MDA = Malonaldehyde.

Table 17. Postmortem Processing Treatment by Breed Interactions for Traits Involving the <i>Longissimus</i> (Ribeye) on Initial day of Processing, After 14 days of Ageing, and After Evaluation of Shelf Life ^z														
Trait	Covariate ^y	Postmortem Processing Treatments ^x By Breed ^x								Contrasts ^w				
		BRIT	CONT	BRIT	CONT	BRIT	CONT	BRIT	CONT	SE	P>F	1	2	3
		NPP	NPP	SS	SS	SS/ME	SS/ME	SS/ME	SS/ME					
Initial Day of Processing														
pH	NA	5.47	5.48	5.44	5.46	5.50	5.47	5.48	5.46	0.015	0.106	0.303	0.036	0.521
14 Day Aged Product														
pH	NA	5.56	5.54	5.53	5.57	5.56	5.50	5.52	5.51	0.022	0.014	0.522	0.002	0.966
L*	A, G	32.0	33.1	33.6	33.5	34.5	34.2	35.8	34.0	0.55	0.031	0.017	0.857	0.064
Shelf Life														
Drip loss 1 ^u , %	NA	2.23	2.05	1.93	1.79	3.10	2.38	2.52	2.98	0.308	0.103	0.844	0.197	0.023
Drip loss 2, %	NA	2.23	2.05	1.93	1.79	3.10	2.38	2.52	2.98	0.308	0.103	0.028 ^t	0.149 ^g	0.916 ^t

^z Initial Day of Processing refers to samples taken after Moisture Enhancement where appropriate (approximately 24 h postmortem for all muscles); 14 Day Aged Product refers to steaks that have been aged at < 4°C until 14 d postmortem; Shelf Life refers to measurements on 18 d aged steaks where 14 d aged steaks are placed on foam trays and overwrapped with PVC film and stored at < 4°C in a chill cooler for an additional 4 d before sampling.

^y Treatment= Either No Postmortem Processing (NPP), Skeletal Separation (SS), Moisture Enhancement (ME), or Skeletal Separation and Moisture Enhancement (SS/ME).

^x Breed represents either predominantly British (BRIT) or predominantly Continental (CONT) animals.

^w Contrast 1 = No Postmortem Processing (NPP) vs. All other Postmortem Processing Treatments by Breed; Contrast 2 = SS vs ME by Breed; Contrast 3 =Combination (SS/ME) versus the average of SS and ME by Breed.

^v Covariates: Age at Slaughter (A) and Grade Fat (G) were included in the model and were noted here when significant at P<0.10. Traits are labeled NA when the P>F value for all covariates is greater than 0.10.

^uDrip loss 1 follows the conventional contrasts listed above; Drip loss 2 includes the contrasts: ^uSS/ME vs. All other PPT, ^gME vs. SS and NPP, ^tSS vs. NPP.

Table 18. Effects of Postmortem Processing Treatments on Traits Involving the <i>Semimembranosus</i> (SM) on Initial day of Processing, After 14 days of Ageing, and After Evaluation of Shelf Life ²									
Trait	Covariate ^x	Postmortem Processing Treatments ¹ (PPT)					Contrasts		
		NPP	SS	ME	SS/ME	SE	P>F	Contrast 1: NPP vs. all other PPT	Contrast 2: SS vs. ME of SS and ME
Initial Day of Processing									
% Solution Retained	A	-	-	9.5	9.9	0.22	0.047	-	-
pH	A	5.46	5.48	5.47	5.46	0.015	0.750	0.486	0.844
L*	G	31.8	31.4	31.8	31.2	0.47	0.651	0.423	0.474
Hue ^w	NA	12.0	11.6	11.7	12.4	0.73	0.779	0.866	0.908
Chroma ^y	NA	23.4	23.4	21.7	22.0	0.46	0.002	0.015	0.310
14 Day Aged Product									
pH	NA	5.52	5.52	5.51	5.50	0.013	0.479	0.372	0.508
L*	NA	34.5	34.4	35.9	34.8	0.53	0.065	0.204	0.016
Hue	NA	15.7	15.6	16.5	15.9	0.75	0.809	0.691	0.364
Chroma	G	24.2	23.8	23.2	23.5	0.35	0.174	0.053	0.232
Shelf Life									
pH	A, G	5.54	5.55	5.59	5.56	0.014	<0.001	0.018	<0.001
L*	G	32.8	32.2	32.8	32.8	0.52	0.720	0.711	0.374
Hue	G	16.6	14.7	19.6	15.9	1.14	0.028	0.908	0.003
Chroma	G	23.4	23.0	24.2	21.8	0.72	0.155	0.553	0.250
Drip loss 1 ^z , %	NA	1.79	1.77	2.26	2.27	0.175	0.001	0.015	0.006
Drip loss 2, %	NA	1.79	1.77	2.26	2.27	0.175	0.001	0.022 ^f	0.002 ^g
TBARS ^u , mg MDA/kg ¹	A	0.59	0.72	0.57	0.53	0.005	0.031	0.799	0.029
² Initial Day of Processing refers to samples taken after Moisture Enhancement where appropriate (approximately 24 h postmortem for all muscles); 14 Day Aged Product refers to steaks that have been aged at < 4°C until 14 d postmortem; Shelf Life refers to measurements on 18 d aged steaks where 14 d aged steaks are placed on foam trays and overwrapped with PVC film and stored at < 4°C in a chill cooler for an additional 4 d before sampling.									
¹ Treatment= Either No Postmortem Processing (NPP), Skeletal Separation (SS), Moisture Enhancement (ME), or Skeletal Separation and Moisture Enhancement (SS/ME).									
^x Covariates; Age at Slaughter (A) and Grade Fat (G) were included in the model and noted here when significant at P<0.10. Traits are labeled NA when the P>F value for all covariates is greater than 0.10.									
^w Hue = Tan ⁻¹ (b*/a*); ^y Chroma = Square root of ((a*) ² + (b*) ²); ^z TBARS = Thiobarbituric Acid Reactive Substances; ¹ MDA = Malonaldehyde.									
^f Drip loss 1 follows the conventional contrasts listed above: Drip loss 2 includes the contrasts: ¹ SS/ME vs. All other PPT; ^g ME vs. SS and NPP; ^z SS vs. NPP.									

² Initial Day of Processing refers to samples taken after Moisture Enhancement where appropriate (approximately 24 h postmortem for all muscles); 14 Day Aged Product refers to steaks that have been aged at < 4°C until 14 d postmortem; Shelf Life refers to measurements on 18 d aged steaks where 14 d aged steaks are placed on foam trays and overwrapped with PVC film and stored at < 4°C in a chill cooler for an additional 4 d before sampling.

¹ Treatment= Either No Postmortem Processing (NPP), Skeletal Separation (SS), Moisture Enhancement (ME), or Skeletal Separation and Moisture Enhancement (SS/ME).

^a Covariates: Age at Slaughter (A) and Grade Fat (G) were included in the model and noted here when significant at P<0.10. Traits are labeled NA when the P>F value for all covariates is greater than 0.10.

^w Hue = Tan⁻¹ (b*/a*); ^v Chroma = Square root of ((a*)² + (b*)²); ^u TBARS = Thiobarbituric Acid Reactive Substances; ¹ MDA = Malonaldehyde.

^s Drip loss 1 follows the conventional contrasts listed above; Drip loss 2 includes the contrasts: ¹SS/ME vs. All other PPT; ^g ME vs. SS and NPP; ^fSS vs. NPP.

Table 19. Effect of Breed on Traits Involving the <i>Semimembranosus</i> (SM) on Initial day of Processing, After 14 days of Ageing, and After Evaluation of Shelf Life ^z					
Trait	Covariate ^x	Breed ^y		SE	P>F
		British	Continental		
Initial Day of Processing					
% Solution Retained	A	9.6	9.8	0.54	0.548
pH	NA	5.47	5.46	0.015	0.666
L*	A, G	31.7	31.4	0.49	0.693
Hue ^w	NA	11.9	12.0	0.88	0.980
Chroma ^v	G	22.6	22.6	0.45	0.948
14 Day Aged Product					
pH	NA	5.52	5.51	0.015	0.709
L*	A, G	35.1	34.7	0.55	0.576
Hue	G	16.1	15.7	0.75	0.703
Chroma	NA	23.3	24.0	0.31	0.114
Shelf Life					
pH	A	5.57	5.55	0.018	0.292
L*	A, G	32.6	32.7	0.50	0.935
Hue	A, G	17.1	16.2	0.86	0.458
Chroma	A, G	23.3	22.9	0.51	0.633
Drip loss, %	NA	2.22	1.82	0.201	0.174
TBARS ^u , mg MDA/kg ^t	NA	0.69	0.52	0.040	0.003

^z Initial Day of Processing refers to samples taken after Moisture Enhancement where appropriate (approximately 24 h postmortem for all muscles); 14 Day Aged Product refers to steaks that have been aged at < 4°C until 14 d postmortem; Shelf Life refers to measurements on 18 d aged steaks where 14 d aged steaks are placed on foam trays and overwrapped with PVC film and stored at < 4°C in a chill cooler for an additional 4 d before sampling.

^y Breed designates cattle with predominantly British or Continental breeding.

^x Covariates, Age at Slaughter (A) and Grade Fat (G) were included in the model and were noted here when significant at P<0.10. Traits are labeled NA when the P>F value for all covariates is greater than 0.10.

^w Hue = Tan⁻¹ (b*/a*); ^v Chroma = Square root of ((a*)² + (b*)²).

^u TBARS = Thiobarbituric Acid Reactive Substances; ^t MDA = Malonaldehyde.

^z Initial Day of Processing refers to samples taken after Moisture Enhancement where appropriate (approximately 24 h postmortem for all muscles); 14 Day Aged Product refers to steaks that have been aged at < 4°C until 14 d postmortem; Shelf Life refers to measurements on 18 d aged steaks where 14 d aged steaks are placed on foam trays and overwrapped with PVC film and stored at < 4°C in a chill cooler for an additional 4 d before sampling.

^y Breed designates cattle with predominantly British or Continental breeding.

^x Covariates; Age at Slaughter (A) and Grade Fat (G) were included in the model and were noted here when significant at P<0.10. Traits are labeled NA when the P>F value for all covariates is greater than 0.10.

^w Hue = $\tan^{-1}(b^*/a^*)$; ^v Chroma = Square root of $((a^*)^2 + (b^*)^2)$.

^u TBARS = Thiobarbituric Acid Reactive Substances; ^t MDA = Malonaldehyde.

Table 20. Postmortem Processing Treatment by Breed Interactions for Traits Involving the <i>Semimembranosus</i> (SM) on Initial day of Processing, After 14 days of Ageing, and After Evaluation of Shelf Life ^z												
Trait	Covariate ^v	Postmortem Processing Treatments ^y By Breed ^x						SE	P>F	Contrasts ^w		
		BRIT	CONT	BRIT	CONT	BRIT	CONT			1	2	3
		NPP	NPP	SS	SS	ME	ME					
Shelf Life												
Hue ^u	A, G	16.0	17.2	14.3	15.1	22.4	16.7	15.9	1.58	0.135	0.049	0.416
^z Initial Day of Processing refers to samples taken after Moisture Enhancement where appropriate (approximately 24 h postmortem for all muscles); 14 Day Aged Product refers to steaks that have been aged at < 4°C until 14 d postmortem; Shelf Life refers to measurements on 18 d aged steaks where 14 d aged steaks are placed on foam trays and overwrapped with PVC film and stored at < 4°C in a chill cooler for an additional 4 d before sampling.												
^y Treatment= Either No Postmortem Processing (NPP), Skeletal Separation (SS), Moisture Enhancement (ME), or Skeletal Separation and Moisture Enhancement (SS/ME).												
^x Breed represents either predominantly British (BRIT) or predominantly Continental (CONT) animals.												
^w Contrast 1 = No Postmortem Processing (NPP) vs. All other Postmortem Processing Treatments by Breed; Contrast 2 = SS vs. ME by Breed; Contrast 3 = Combination (SS/ME) versus the average of SS and ME by Breed.												
^v Covariates: Age at Slaughter (A) and Grade Fat (G) were included in the model and were noted here when significant at P<0.10. Traits are labeled NA when the P>F value for all covariates is greater than 0.10.												
^u Hue = $\tan^{-1}(b^*/a^*)$												

Trait	Covariate ^x	Postmortem Processing Treatments ^y (PPT)					Contrasts			
		NPP	SS	ME	SS/ME	SE	P>F	Contrast 1: NPP vs. all other PPT	Contrast 2: SS vs. ME	Contrast 3: SS/ME vs. average of SS and ME
Initial Day of Processing										
% Solution Retained	A, G	-	-	9.0	9.5	0.27	0.176	-	-	-
pH	A	5.49	5.50	5.52	5.50	0.016	0.445	0.284	0.351	0.344
L*	A, G	33.4	33.4	33.3	33.3	0.40	0.994	0.832	0.898	0.907
Hue ^w	NA	12.1	12.0	11.8	12.1	0.69	0.950	0.705	0.701	0.762
Chroma ^v	G	21.9	21.6	20.0	20.2	0.32	<0.001	<0.001	<0.001	0.032
14 Day Aged Product										
pH	NA	5.53	5.53	5.50	5.50	0.016	0.040	0.046	0.045	0.530
L*	NA	36.0	35.2	36.5	36.3	0.47	0.032	0.816	0.009	0.328
Hue	G	17.5	16.3	17.5	17.4	0.67	0.167	0.408	0.082	0.398
Chroma	NA	23.1	22.2	21.9	22.2	0.30	0.008	<0.001	0.488	0.610
Shelf Life										
pH	NA	5.58	5.59	5.56	5.59	0.021	0.627	0.963	0.269	0.379
L*	A, G	34.9	34.2	35.7	34.9	0.43	0.008	0.826	<0.001	0.923
Hue	NA	17.7	17.6	19.3	18.7	0.49	0.003	0.034	0.002	0.476
Chroma	A, G	22.7	22.7	22.0	23.0	0.33	0.153	0.776	0.132	0.065
Drip loss 1 ^s , %	NA	1.71	1.63	2.30	1.73	0.143	0.001	0.152	<0.001	0.097
Drip loss 2, %	NA	1.71	1.63	2.30	1.73	0.143	0.001	0.245 ^t	<0.001 ^q	0.590 ^p
APC, cfu	NA	19	13	13	13	2.5	0.930	0.569	0.781	0.871
APC, Log cfu/cm ²	NA	0.89	0.96	0.96	0.98	0.921	0.603	0.513	0.305	0.429
TBARS ^u , mg MDA/kg ^t	A	0.74	0.71	0.56	0.61	0.006	0.086	0.073	0.062	0.761

^z Initial Day of Processing refers to samples taken after Moisture Enhancement where appropriate (approximately 24 h postmortem for all muscles), 14 Day Aged Product refers to steaks that have been aged at < 4°C until 14 d postmortem; Shelf Life refers to measurements on 18 d aged steaks where 14 d aged steaks are placed on foam trays and overwrapped with PVC film and stored at < 4°C in a chill cooler for an additional 4 d before sampling.

^y Treatment= Either No Postmortem Processing (NPP), Skeletal Separation (SS), Moisture Enhancement (ME), or Skeletal Separation and Moisture Enhancement (SS/ME).

^x Covariates; Age at Slaughter (A) and Grade Fat (G) were included in the model and noted here when significant at P<0.10. Traits are labeled NA when the P>F value for all covariates is greater than 0.10.

^w Hue = Tan⁻¹ (b*/a*); Chroma = Square root of ((a*)² + (b*)²);^u TBARS = Thiobarbituric Acid Reactive Substances; ^tMDA = Malonaldehyde.

^pDrip loss 1 follows the conventional contrasts listed above. Drip loss 2 includes the contrasts: ^sSS/ME vs. All other PPT. ^qME vs. SS and NPP. ^rSS vs. NPP.

^z Initial Day of Processing refers to samples taken after Moisture Enhancement where appropriate (approximately 24 h postmortem for all muscles), 14 Day Aged Product refers to steaks that have been aged at < 4°C until 14 d postmortem; Shelf Life refers to measurements on 18 d aged steaks where 14 d aged steaks are placed on foam trays and overwrapped with PVC film and stored at < 4°C in a chill cooler for an additional 4 d before sampling.

^y Treatment= Either No Postmortem Processing (NPP), Skeletal Separation (SS), Moisture Enhancement (ME), or Skeletal Separation and Moisture Enhancement (SS/ME).

^x Covariates: Age at Slaughter (A) and Grade Fat (G) were included in the model and noted here when significant at P<0.10. Traits are labeled NA when the P>F value for all covariates is greater than 0.10.

^w Hue = Tan⁻¹ (b*/a*), Chroma = Square root of ((a*)² + (b*)²),^u TBARS = Thiobarbituric Acid Reactive Substances; ^t MDA = Malonaldehyde.

^s Drip loss 1 follows the conventional contrasts listed above; Drip loss 2 includes the contrasts: ^vSS/ME vs. All other PPT; ^q ME vs. SS and NPP; ^pSS vs. NPP.

Table 22. Effect of Breed on Traits Involving the <i>Semiteindinosus</i> (ST) on Initial day of Processing, After 14 days of Ageing, and After Evaluation of Shelf Life ^z					
		Breed ^y			
Trait	Covariate ^x	British	Continental	SE	P>F
Initial Day of Processing					
% Solution Retained	A	9.3	9.1	0.29	0.700
pH	NA	5.52	5.49	0.017	0.258
L*	A, G	33.2	33.5	0.43	0.636
Hue ^w	NA	11.2	12.8	0.78	0.154
Chroma ^v	G	20.8	21.1	0.30	0.437
Sarcomere Length, μ m	NA	2.42	2.39	0.050	0.668
14 Day Aged Product					
pH	NA	5.53	5.50	0.019	0.397
L*	A, G	35.7	36.3	0.53	0.455
Hue	G	16.6	17.8	0.76	0.272
Chroma	NA	21.9	22.8	0.31	0.048
Shelf Life					
pH	A	5.60	5.56	0.024	0.225
L*	A, G	34.6	35.2	0.50	0.450
Hue	A, G	18.2	18.5	0.54	0.724
Chroma	A, G	22.3	22.8	0.27	0.194
Drip loss, %	NA	1.98	1.71	0.147	0.204
APC, cfu/ cm ²	NA	16	11	3.2	0.275
APC, Log cfu/cm ²	G	1.03	0.81	0.080	0.077
TBARS ^u , mg MDA/kg ^l	NA	0.73	0.58	0.052	0.048
^z Initial Day of Processing refers to samples taken after Moisture Enhancement where appropriate (approximately 24 h postmortem for all muscles); 14 Day Aged Product refers to steaks that have been aged at < 4°C until 14 d postmortem; Shelf Life refers to measurements on 18 d aged steaks where 14 d aged steaks are placed on foam trays and overwrapped with PVC film and stored at < 4°C in a chill cooler for an additional 4 d before sampling.					
^y Breed designates cattle with predominantly British or Continental breeding.					
^x Covariates; Age at Slaughter (A) and Grade Fat (G) were included in the model and were noted here when significant at P<0.10. Traits are labeled NA when the P>F value for all covariates is greater than 0.10.					
^w Hue = Tan ⁻¹ (b*/a*); ^v Chroma = Square root of ((a* ² + b* ²)); ^u TBARS = Thiobarbituric Acid Reactive Substances; ^l MDA = Malonaldehyde.					

^z Initial Day of Processing refers to samples taken after Moisture Enhancement where appropriate (approximately 24 h postmortem for all muscles); 14 Day Aged Product refers to steaks that have been aged at < 4°C until 14 d postmortem; Shelf Life refers to measurements on 18 d aged steaks where 14 d aged steaks are placed on foam trays and overwrapped with PVC film and stored at < 4°C in a chill cooler for an additional 4 d before sampling.

^y Breed designates cattle with predominantly British or Continental breeding.

^x Covariates: Age at Slaughter (A) and Grade Fat (G) were included in the model and were noted here when significant at P<0.10. Traits are labeled NA when the P>F value for all covariates is greater than 0.10.

^w Hue = $\tan^{-1} (b^*/a^*)$; ^v Chroma = Square root of $((a^*)^2 + (b^*)^2)$; ^u TBARS = Thiobarbituric Acid Reactive Substances; ^l MDA = Malonaldehyde.

Table 23. Postmortem Processing Treatment by Breed Interactions for Traits Involving the <i>Semiteminosus</i> (ST) on Initial day of Processing, After 14 days of Ageing, and After Evaluation of Shelf Life ^z														
Trait	Covariate ^y	Postmortem Processing Treatments ^x By Breed ^w								Contrasts ^w				
		BRIT NPP	CONT NPP	BRIT SS	CONT SS	BRIT ME	CONT ME	BRIT SS/ME	CONT SS/ME	SE	P>F	1	2	3
Shelf Life														
Drip loss 1 ^u , %	NA	1.79	1.64	1.77	1.50	2.70	1.90	1.50	1.67	0.180	0.075	0.473	0.130	0.027
Drip loss 2, %	NA	1.79	1.64	1.77	1.50	2.70	1.90	1.50	1.67	0.180	0.075	0.058 ^t	0.051 ^s	0.688 ^t
^z Initial Day of Processing refers to samples taken after Moisture Enhancement where appropriate (approximately 24 h postmortem for all muscles); 14 Day Aged Product refers to steaks that have been aged at < 4°C until 14 d postmortem; Shelf Life refers to measurements on 18 d aged steaks where 14 d aged steaks are placed on foam trays and overwrapped with PVC film and stored at < 4°C in a chill cooler for an additional 4 d before sampling.														
^y Treatment= Either No Postmortem Processing (NPP), Skeletal Separation (SS), Moisture Enhancement (ME), or Skeletal Separation and Moisture Enhancement (SS/ME).														
^x Breed represents either predominantly British (BRIT) or predominantly Continental (CONT) animals.														
^w Contrast 1 = No Postmortem Processing (NPP) vs. All other Postmortem Processing Treatments by Breed; Contrast 2 = SS vs. ME by Breed; Contrast 3 =Combination (SS/ME) versus the average of SS and ME by Breed.														
^v Covariates: Age at Slaughter (A) and Grade Fat (G) were included in the model and were noted here when significant at P<0.10. Traits are labeled NA when the P>F value for all covariates is greater than 0.10.														
^u Drip loss 1 follows the conventional contrasts listed above; Drip loss 2 includes the contrasts: ^s SS/ME vs. All other PPT, ^s ME vs. SS and NPP, ^s SS vs. NPP.														

Shear Force		Postmortem Processing Treatments ²						Contrasts		
Muscle	NPP	SS	ME	SS/ME	Avg. SE	P>F	Contrast 1: NPP vs. all other PPT	Contrast 2: SS vs. ME	Contrast 3: SS/ME vs. average of SS and ME	
Longissimus - Loin	4.99	4.53	4.35	3.65	0.199	<0.001	<0.001	0.398	<0.001	
Longissimus - Ribeye	5.26	4.68	4.32	3.77	0.211	<0.001	<0.001	0.151	0.001	
<i>Semimembranosus</i> (SM)	6.32	6.23	5.63	5.60	0.157	<0.001	<0.001	<0.001	0.019	
<i>Semitendinosus</i> (ST)	5.93	6.01	5.73	5.60	0.122	0.029	0.208	0.071	0.052	
Cookloss (%)		Postmortem Processing Treatments ²						Contrasts		
Muscle	NPP	SS	ME	SS/ME	Avg. SE	P>F	Contrast 1: NPP vs. all other PPT	Contrast 2: SS vs. ME	Contrast 3: SS/ME vs. average of SS and ME	
Longissimus - Loin	19.67	18.63	19.77	21.69	0.485	<0.001	0.482	0.097	<0.001	
Longissimus - Ribeye	20.05	19.75	21.07	22.22	0.599	0.008	0.110	0.109	0.012	
<i>Semimembranosus</i> (SM)	24.52	25.09	25.48	25.64	0.454	0.281	0.055	0.572	0.535	
<i>Semitendinosus</i> (ST)	23.36	23.78	24.65	25.43	0.572	0.052	0.040	0.343	0.103	

²Treatments=No Postmortem Processing (NPP); Skeletal Separation (SS); Moisture Enhancement (ME); Skeletal Separation and Moisture Enhancement (SS/ME).

² Treatments=No Postmortem Processing (NPP); Skeletal Separation (SS); Moisture Enhancement (ME); Skeletal Separation and Moisture Enhancement (SS/ME).

Table 25: Effects of Postmortem Ageing on Shear Force and Cook Loss Measurements							
Shear Force (Kg)	Postmortem Ageing (days)			SE	P > F	Contrasts	
	7	14	21			7 vs the average of 14 and 21 days ageing	14 vs. 21 days ageing
Muscle							
Longissimus - Loin	4.81	4.28	4.06	0.161	<0.001	<0.001	0.022
Longissimus - Ribeye	4.86	4.47	4.19	0.161	<0.001	<0.001	0.001
Semimembranosus (SM)	6.00	6.02	5.81	0.140	0.110	0.425	0.056
Semitendinosus (ST)	6.75	5.36	5.35	0.098	<0.001	<0.001	0.937
Cook loss (%)	Postmortem Ageing (days)			SE	P > F	Contrasts	
	7	14	21			7 vs the average of 14 and 21 days ageing	14 vs. 21 days ageing
Muscle							
Longissimus - Loin	20.9	19.3	19.7	0.39	0.008	0.003	0.001
Longissimus - Ribeye	21.7	20.9	19.8	0.50	0.007	0.009	0.077
Semimembranosus (SM)	26.0	24.9	24.7	0.36	0.020	0.006	0.774
Semitendinosus (ST)	25.3	24.2	23.5	0.45	0.001	0.005	0.241

Table 26: Postmortem Processing Treatment by Postmortem Ageing Interactions on Shear Force and Cook loss Measurements														
Shear Force (Kg)	Muscle	Days Ageing	Postmortem Processing Treatments ²				SE	P>F	Contrasts ³					
			NPP	SS	ME	SS/ME			1	2	3	4	5	6
Longissimus (Loin)		7	5.71	4.87	4.83	3.82	0.229	0.015	0.003	0.333	0.379	0.560	0.111	0.158
		14	4.84	4.36	4.18	3.77								
		21	4.45	4.38	4.04	3.35								
Longissimus (Ribeye)		7	5.90	4.90	4.65	4.00	0.232	0.002	<0.001	0.022	0.381	0.193	0.654	0.961
		14	5.23	4.74	4.16	3.76								
		21	4.64	4.41	4.14	3.59								
Cook Loss (%)	Muscle	Days Ageing	Postmortem Processing Treatments ²				SE	P>F	Contrasts ³					
			NPP	SS	ME	SS/ME			1	2	3	4	5	6
Semitendinosus (ST)		7	25.52	25.01	25.72	24.82	0.883	0.114	0.031	0.848	0.871	0.883	0.040	0.248
		14	22.54	23.20	24.27	26.68								
		21	22.03	23.15	23.97	24.80								

²Treatments=No Postmortem Processing (NPP); Skeletal Separation (SS); Moisture Enhancement (ME); Skeletal Separation and Moisture Enhancement (SS/ME).

³Contrast 1 = Interaction of No Postmortem Processing (NPP) vs. all other Postmortem Processing Treatments by 7 vs. 14 and 21 d ageing; Contrast 2 = Interaction of No Postmortem Processing (NPP) vs. all other Postmortem Processing Treatments by 14 vs. 21 d ageing; Contrast 3 = Interaction of SS vs. ME by 7 vs. 14 and 21 d ageing; Contrast 4 = Interaction of SS vs. ME by 14 vs 21 d ageing; Contrast 5 = Interaction of SS/ME vs. the average of SS and ME by 7 vs. 14 and 21 d ageing; Contrast 6 = Interaction of SS/ME vs. the average of SS and ME by 14 vs. 21 d ageing.

² Treatments=No Postmortem Processing (NPP); Skeletal Separation (SS); Moisture Enhancement (ME); Skeletal Separation and Moisture Enhancement (SS/ME).

³ Contrast 1 = Interaction of No Postmortem Processing (NPP) vs. all other Postmortem Processing Treatments by 7 vs. 14 and 21 d ageing; Contrast 2 = Interaction of No Postmortem Processing (NPP) vs. all other Postmortem Processing Treatments by 14 vs. 21 d ageing; Contrast 3 = Interaction of SS vs. ME by 7 vs. 14 and 21 d ageing; Contrast 4 = Interaction of SS vs. ME by 14 vs. 21 d ageing; Contrast 5 = Interaction of SS/ME vs. the average of SS and ME by 7 vs. 14 and 21 d ageing; Contrast 6 = Interaction of SS/ME vs. the average of SS and ME by 14 vs. 21 d ageing.

Traits	Postmortem Processing Treatments for Cull Cow Beef								Red Branded Product					
	NPP ²				SS ³				ME ^x		SS/ME ^w			
	BRIT	CONT	BRIT	CONT	BRIT	CONT	BRIT	CONT	BRIT	CONT	AA ^v	AAA ^u	SE	P>F
Softness	5.32	5.28	5.80	6.28	6.76	6.43	7.31	7.42	7.43	7.43	7.84	7.84	0.482	<0.001
Tenderness	4.77	4.95	5.70	5.84	6.26	5.99	6.83	7.13	7.38	7.38	7.69	7.69	0.486	<0.001
Chewiness	4.31	4.77	5.12	5.30	6.25	5.80	6.60	6.62	7.18	7.18	7.46	7.46	0.494	<0.001
Rate of Breakdown	4.40	4.96	5.33	5.60	5.94	5.84	6.36	6.68	7.18	7.18	7.48	7.48	0.462	<0.001
Initial Juiciness	4.01	3.36	4.43	3.46	5.68	4.17	4.39	4.62	2.73	3.68	3.68	3.68	0.589	<0.001
Overall Juiciness	3.98	3.48	4.26	3.50	5.51	4.14	4.05	4.40	3.06	3.68	3.68	3.68	0.576	<0.001
Flavour	4.32	4.33	4.14	4.30	4.40	4.26	4.25	4.18	3.51	4.10	4.10	4.10	0.674	0.049
Off-flavour	1.19	1.03	1.03	1.17	1.72	1.72	1.99	2.04	1.57	1.74	1.74	1.74	0.424	0.042

Contrasts	Contrast 1: No Postmortem Processing (NPP) vs. all other Postmortem Processing Treatments	Contrast 2: SS vs. ME	Contrast 3: SS/ME vs. the average of SS and ME	Contrast 4: AA and AAA product vs. SS/ME	Contrast 5: AA vs. AAA product	Contrast 6: Interaction of No Postmortem Processing (NPP) vs. all other Postmortem Processing Treatments between breeds	Contrast 7: Interaction of SS vs. ME between breeds	Contrast 8: Interaction of SS/ME vs. the average of SS and ME treatments between breeds	Contrast 9: British vs. Continental
Softness	<0.001	0.136	0.001	0.460	0.429	0.792	0.242	0.949	0.887
Tenderness	<0.001	0.314	0.001	0.143	0.547	0.802	0.543	0.467	0.821
Chewiness	<0.001	0.021	0.001	0.051	0.576	0.251	0.336	0.745	0.880
Rate of Breakdown	<0.001	0.274	0.011	0.032	0.551	0.458	0.614	0.670	0.461
Initial Juiciness	0.005	0.008	0.824	<0.001	0.044	0.852	0.437	0.009	0.033
Overall Juiciness	0.020	0.004	0.636	0.005	0.128	0.843	0.327	0.005	0.043
Flavour	0.673	0.611	0.736	0.031	0.023	0.945	0.482	0.811	0.951
Off-flavour	0.028	0.033	0.017	0.159	0.595	0.479	0.732	0.944	0.975

² No Postmortem Processing Treatment.
³ Skeletal Separation (MOLAS).
^x Moisture Enhancement.
^w Moisture Enhancement and Skeletal Separation.
^v Denotes Grade AA Steaks with no postmortem processing.
^u Denotes Grade AAA Steaks with no postmortem processing.

Traits	Postmortem Processing Treatments for Cull Cow Beef									Red Branded Product				
	NPP ^z			SS ^y			ME ^x			SS/ME ^w			Marbling Grade	
	BRIT	CONT	BRIT	CONT	BRIT	CONT	BRIT	CONT	BRIT	CONT	AA ^v	AAA ^u	SE	P>F
Softness	4.06	4.78	4.69	5.24	5.10	5.52	4.60	5.70	4.60	5.70	6.27	6.87	0.541	<0.001
Tenderness	4.14	4.62	4.34	5.12	4.76	5.19	4.48	5.50	4.48	5.50	6.11	6.96	0.576	<0.001
Chewiness	4.35	4.74	4.69	5.27	5.09	5.54	4.47	5.59	4.47	5.59	6.11	7.04	0.596	<0.001
Rate of Breakdown	4.60	4.90	5.03	5.40	5.10	5.57	4.64	5.65	4.64	5.65	6.14	7.04	0.583	<0.001
Initial Juiciness	3.34	3.27	4.69	4.23	2.98	4.00	4.27	3.65	4.27	3.65	3.21	2.33	0.639	0.041
Overall Juiciness	3.38	3.26	4.88	4.17	3.17	3.97	4.44	3.76	4.44	3.76	3.28	2.52	0.611	0.022
Flavour	5.10	4.86	5.33	4.62	4.33	4.51	4.91	4.40	4.91	4.40	3.96	4.12	0.711	<0.001
Off-flavour	1.03	1.24	1.64	1.33	1.92	2.06	2.3	2.22	2.3	2.22	1.83	1.32	0.382	0.006
Contrasts	Contrast 1: No Postmortem Processing (NPP) vs. all other Postmortem Processing Treatments	Contrast 2: SS vs. ME	Contrast 3: SS/ME vs. the average of SS and ME	Contrast 4: AA and AAA product vs. SS/ME	Contrast 5: AA vs. AAA product	Contrast 6: Interaction of No Postmortem Processing (NPP) vs. all other Postmortem Processing Treatments between breeds	Contrast 7: Interaction of SS vs. ME between breeds	Contrast 8: Interaction of SS/ME vs. the average of SS and ME treatments between breeds	Contrast 9: British vs. Continental					
Softness	0.015	0.376	0.971	<0.001	0.252	0.949	0.869	0.317	0.068					
Tenderness	0.083	0.526	0.680	<0.001	0.096	0.643	0.658	0.485	0.064					
Chewiness	0.050	0.374	0.702	<0.001	0.058	0.503	0.840	0.237	0.063					
Rate of Breakdown	0.090	0.746	0.695	<0.001	0.053	0.506	0.879	0.242	0.080					
Initial Juiciness	0.112	0.076	0.972	0.015	0.184	0.960	0.183	0.292	0.937					
Overall Juiciness	0.050	0.052	0.900	0.007	0.204	0.918	0.127	0.337	0.643					
Flavour	0.162	0.046	0.845	0.012	0.605	0.639	0.023	0.437	0.025					
Off-flavour	0.001	0.087	0.041	0.011	0.129	0.357	0.295	0.990	0.945					

^z No Postmortem Processing Treatment.

^y Skeletal Separation (MOLAS).

^w Moisture Enhancement.

^v Moisture Enhancement and Skeletal Separation.

^u Denotes Grade AA Steaks with no postmortem processing.

^a Denotes Grade AAA Steaks with no postmortem processing.

^z No Postmortem Processing Treatment.

^y Skeletal Separation (MOLAS).

^x Moisture Enhancement.

^w Moisture Enhancement and Skeletal Separation.

^v Denotes Grade AA Steaks with no postmortem processing.

^u Denotes Grade AAA Steaks with no postmortem processing.

4. REFERENCES

- Aalhus, J. L., D. R. Best, F. Costello, and L. E. Jeremiah. 1999. A simple, on-line processing method for improving beef tenderness. *Can. J. Anim. Sci.* 79(1): 27-34.
- Aalhus, J. L., M. E. R. Dugan, W. M. Robertson, D. R. Best, and I. L. Larsen. 2004. A within-animal examination of postmortem ageing for up to 21 d on tenderness in the bovine *longissimus* thoracis and *semimembranosus* muscles. *Can. J. Anim. Sci.* 84(2): 301-304.
- Aktas, N., M. I. Aksu, and M. Kaya. 2003. The effect of organic acid marination on tenderness, cooking loss and bound water content of beef. *J. Muscle Foods.* 14(3): 181-194.
- Aktas, N., and M. Kaya. 2001. The influence of marinating with weak organic acids and salts on the intramuscular connective tissue and sensory properties of beef. *Eur. Food Res. Technol.* 213(2): 88-94.
- Alarcon-Rojo A., and E. Dransfield. 1989. Effect of calcium ions on texture of beef during conditioning. *Proceedings, Intl. Meat Sci. Tech.* 35(3): 1141-1144.
- Alarcon-Rojo, A., and E. Dransfield. 1995. Alteration of postmortem aging in beef by the addition of enzyme-inhibitors and activators. *Meat Sci.* 41(2): 163-178.
- Ali, M. S., H. S. Yang, J. Y. Jeong, S. H. Moon, Y. H. Hwang, G. B. Park et al. 2008. Effect of chilling temperature of carcass on breast meat quality of duck. *Poult. Sci.* 87(9): 1860-1867.
- AMSA. 1995. Research guidelines for cookery, sensory evaluation, and instrumental tenderness measurements of fresh meat. Savoy, IL: American Meat Science Association and National Live Stock and Meat Board.
- Anderson, M. E., and R. T. Marshall. 1990. Reducing microbial populations on beef tissues: Concentration and temperature of an acid mixture. *J. Food Sci.* 55(4): 903-905.
- Apple, J.K. 1999. Influence of body condition score on live and carcass value of cull cow beef cows. *J. Anim. Sci.* 77(10): 2610-2620.
- Apple, J. K., E. B. Kegley, D. L. Galloway, T. J. Wistuba, and L. K. Rakes. 2005. Duration of restraint and isolation stress as a model to study the dark-cutting condition in cattle. *J. Anim. Sci.* 83(5): 1202-1214.
- Ashmore, C. R., L. Doerr, G. Foster, and F. Carroll. 1971. Respiration of mitochondria isolated from dark-cutting beef. *J. Anim. Sci.* 33(3): 574-577.
- Baohua, K., Xinping, D., and Y. L. Xiong. 2006. Postmortem calcium chloride injection alters ultrastructure and improves tenderness of mature Chinese Yellow cattle *longissimus* muscle. *J. Food Sci.* 71(3): 124-129.
- Bartos, L., C. Franc, D. Rehak, and Stipkova. 1993. A practical method to prevent dark-cutting (DFD) in beef. *Meat Sci.* 34(3): 275-282.

- Baublits, R. T., F. W. Pohlman, A. H. Brown, and Z. B. Johnson. 2005. Effects of sodium chloride, phosphate type and concentration, and pump rate on beef biceps femoris quality and sensory characteristics. *Meat Sci.* 70(2): 205-214.
- Baublits, R. T., F. W. Pohlman, A. H. Brown, and Z. B. Johnson. 2006a. Enhancement with varying phosphate types, concentrations, and pump rates, without sodium chloride on beef biceps femoris quality and sensory characteristics. *Meat Sci.* 72(3): 404-414.
- Baublits, R. T., F. W. Pohlman, A. H. Brown, E. J. Yancey, and Z. B. Johnson. 2006b. Impact of muscle type and sodium chloride concentration on the quality, sensory, and instrumental color characteristics of solution enhanced whole-muscle beef. *Meat Sci.* 72(4): 704-712.
- Beatty, S. L., J. K. Apple, L. K. Rakes, and D. L. Kreider. 1999. Early postmortem skeletal alterations effect on sarcomere length, myofibrillar fragmentation, and muscle tenderness of beef from light-weight, Brangus heifers. *J. Muscle Foods.* 10(1): 67-78.
- Bendall, J. R. 1954. The swelling effect of polyphosphates on lean meat. *J. Sci. Food Agric.* 5(10): 468-475.
- Bendall, J. R. 1960. In the Structure and Function of Muscle, Vol. III. Academic Press: New York, NY.
- Berge, P., P. Ertbjerg, L. M. Larsen, T. Astruc, X. Vignon, and A. J. Moller. 2001. Tenderization of beef by lactic acid injected at different times post mortem. *Meat Sci.* 57(4): 347-357.
- Bishop, M. D., M. Koohmaraie, J. Killefer, and S. Kappes. 1993. Rapid communication: Restriction fragment length polymorphisms in the bovine calpastatin gene. *J. Anim. Sci.* 71(8): 2277.
- Bohaychuk, V. M., and G. G. Greer. 2003. Bacteriology and storage life of moisture-enhanced pork. *J. Food Prot.* 66(2): 293-299.
- Boleman, S. J., S. L. Boleman, R. K. Miller, J. F. Taylor, H. R. Cross, T. L. Wheeler, M. Koohmaraie, S. D. Shackelford, M. F. Miller, R. L. West et al. 1997. Consumer evaluation of beef of known categories of tenderness. *J. Anim. Sci.* 75: 1521-1524.
- Boles, J. A., and P. J. Shand. 2001. Meat cut and injection level affects the tenderness and cook yield of processed roast beef. *Meat Sci.* 59: 259-265.
- Boles, J. A., and J. E. Swan. 1997. Effects of brine ingredients and temperature on cook yields and tenderness of pre-rigor processed roast beef. *Meat Sci.* 45: 87-97.
- Botsoglou, N. A., D. J. Fletouris, G. E. Papageorgiou, V. N. Vassilopoulos, A. J. Mantis, and A. G. Trakatellis. 1994. Rapid, sensitive, and specific thiobarbituric acid method for measuring lipid-peroxidation in animal tissue, food, and feedstuff samples. *J. Agric. Food Chem.* 42(9): 1931-1937.
- Brewer, M. S., J. Jensen, C. Prestat, L. G. Zhu, and F. K. McKeith. 2002. Visual acceptability and consumer purchase intent of enhanced pork loin roasts. *J. Muscle Foods.* 13(1): 53-68.

- Broadway, M.J. 2008. The social representation and reality of BSE's impact in North Central Alberta. *Can. Geog.* 52(4): 451-465.
- Brotsky, E. 1976. Automatic injection of chicken parts with polyphosphate. *Poult. Sci.* 55(2): 653-660.
- Bruce, H. L., J. L. Stark, and S. L. Beilken. 2004. The effects of finishing diet and postmortem ageing on the eating quality of the *M-longissimus* thoracis of electrically stimulated Brahman steer carcasses. *Meat Sci.* 67(2): 261-268.
- Campo, M. M., G. R. Nute, S. I. Hughes, M. Enser, J. D. Wood, and R. I. Richardson. 2006. Flavour perception of oxidation in beef. *Meat Sci.* 72(2): 303-311.
- Carmack, C. F., C. L. Kastner, M. E. Dikeman, J. R. Schwenke, and C. M. G. Zepeda. 1995. Sensory evaluation of beef-flavor intensity, tenderness, and juiciness among major muscles. *Meat Sci.* 39(1): 143-147.
- Carpenter, C. E., D. P. Cornforth, and D. Whittier. 2001. Consumer preferences for beef color and packaging did not affect eating satisfaction. *Meat Sci.* 57: 359-363.
- CBGA. 2010. The Canadian Beef Grading Agency. Quality Grading. Calgary, Alberta. <http://www.beefgradingagency.ca/index.html>. Accessed 08/06/2010
- Cheng, J. H., and H. W. Ockerman. 2007. Effects of phosphate, ascorbic acid, α -tocopherol and salt with nonvacuum tumbling on lipid oxidation and warmed-over flavor of roast beef. *J. Muscle Foods.* 18(3): 313-329.
- Claus, J. R., H. Wang, and N. Marriott. Tenderness improvement through prerigor muscle stretching of Holstein cow carcasses. Conference Proceedings. Indianapolis, IN (USA).
- Claus, J. R., H. Wang, and N. G. Marriott. 1997. Prerigor carcass muscle stretching effects on tenderness of grain-fed beef under commercial conditions. *J. Food Sci.* 62(6): 1231-1234.
- Craig, J. A., J. A. Bowers, X. Y. Wang, and P. A. Seib. 1996. Inhibition of lipid oxidation in meats by inorganic phosphate and ascorbate salts. *J. Food Sci.* 61(5): 1062-1067.
- Cundiff, L.V., F. Szabo, K.E. Gregory, R.M. Koch, M.E. Dikeman, and J.D. Crouse. 1993. Breed comparisons in the Germplasm Evaluation Program at MARC. *Proc. Beef Improv. Fed. Ann. Res. Sym. and Ann. Mtg.* pp. 124-136.
- DeYonge-Freeman, K. G., T. D. Pringle, A. E. Reynolds, and S. E. Williams. 2000. Evaluation of calcium chloride and spice marination on the sensory and textural characteristics of precooked *semitendinosus* roasts. *J. Food Qual.* 23(1): 1-13.
- Dhanda, J. S., R. B. Pegg, J. A. M. Janz, J. L. Aalhus, and P. J. Shand. 2002. Palatability of bison *semimembranosus* and effects of marination. *Meat Sci.* 62: 19-26.
- Diles, J. J. B., M. F. Miller, and B. L. Owen. 1994. Calcium-chloride concentration, injection time, and aging period effects on tenderness, sensory, and retail color attributes of loin steaks from mature cows. *J. Anim. Sci.* 72(8): 2017-2021.
- Dransfield, E. 2008. The taste of fat. *Meat Sci.* 80(1): 37-42.

- Dransfield, E., M. J. Ledwith, and A. A. Taylor. 1991. Effect of electrical stimulation, hip suspension and ageing on quality of chilled pig meat. *Meat Sci.* 29(2) : 129-139.
- Dransfield, E., J. F. Martin, D. Bauchart, S. Abouelkaram, J. Lepetit, J. Culioli et al. 2003. Meat quality and composition of three muscles from French cull cows and young bulls. *J. Anim. Sci.* 76: 387-399.
- Djenane, D., L. Martinez, D. Blanco, J. Yanguela, J. A. Beltran, and P. Roncales. 2005. Effect of lactic acid bacteria on extension of shelf life and growth of *listeria monocytogenes* in beef steaks stored in CO₂-rich atmosphere. *Brazilian J. Microbiol.* 36(4): 405-412.
- Du, M., Aalhus, J. L., Ahn, D. U., Cantino, M. E., Flann, K.L., Geesink, G. H., Clare, A. G., Greaser, M. L., Hausman, G. J., Hopkins, D. L., Lee, E. J., Mancini, R. A., McCormick, R. J., Means, W. J., Nam, K. C., Oksbjerg, N., Poulos, S. P., Rathbone, C. R., Regenstein, J. M., Rhoads, R. P., Riggs, P. K., Robertson, W. M., Rule, D. C., Shen, Q. W., Swartz, D. R., Therkildsen, M., Tong, J. F., Underwood, K. R., Ye, J., Zhou, P. 2009. *Applied Muscle Biology and Meat Science*. CRC Press, Taylor and Francis Group, LLC.
- Eichenberger, B., H. P. Pfirter, C. Wenk, and S. Gebert. 2004. Influence of dietary vitamin E and C supplementation on vitamin E and C content and thiobarbituric acid reactive substances (TBARS) in different tissues of growing pigs. *Archives of Animal Nutrition.* 58(3): 195-208.
- Eikelenboom, G., V. M. H., Barnier, A. H., Hoving-Bolink, F. J. M., Smulders, and J. Culioli. 1998. Effect of pelvic suspension and cooking temperature on the tenderness of electrically stimulated and aged beef, assessed with shear and compression tests. *Meat Sci.* 49(1): 89-99.
- Eilers, J. D., J. B. Morgan, A. M. Martin, R. K. Miller, D. S. Hale, G. R. Acuff et al. 1994. Evaluation of calcium chloride and lactic acid injection on chemical, microbiological and descriptive attributes of mature cow beef. *Meat Sci.* 38(3): 443-451.
- Faustman, C., and Darl Swartz. 2007. *Meat Processing Technology Series: Basic Meat Chemistry*. American Meat Science Association, Savoy, IL.
- Fung, D. Y., M. N. Hajmeer, C. L. Kastner, J. J. Kastner, J. L. Marsden, K. P. Penner, R. K. Phebus, J. S. Smith and M. A. Vanier. 2001. *Meat Safety*. Excerpt from: Hui, Y. H., Nip, W., Rogers, R. W. and O. A. Young. 2001. *Meat Science and Applications*. Marcel Dekker inc., New York, NY.
- Garcia, L. G., K. L. Nicholson, T. W. Hoffman, T. E. Lawrence, D. S. Hale, D. B. Griffin et al. 2008. National beef quality audit-2005: Survey of targeted cattle and carcass characteristics related to quality, quantity, and value of fed steers and heifers. *J. Anim. Sci.* 86(12): 3533-3543.
- Gatellier, P., C. Hamelin, Y. Durand, and M. Renner. 2001. Effect of a dietary vitamin E supplementation on colour stability and lipid oxidation of air- and modified atmosphere-packaged beef. *Meat Sci.* 59(2): 133-140.

- Geesink, G. H., S. Kuchay, A. H. Chishti, and M. Koohmaraie. 2006. Calpain is essential for postmortem proteolysis of muscle proteins [electronic resource]. *J. Anim. Sci.* 84: 2834-2840.
- Gimeno, O., I. Astiasaran, and J. Bello. 2001. Calcium ascorbate as a potential partial substitute for NaCl in dry fermented sausages: Effect on colour, texture and hygienic quality at different concentrations. *Meat Sci.* 57(1): 23-29.
- Gill, C. O., and C. Landers. 2003. Microbiological effects of carcass decontaminating treatments at four beef packing plants. *Meat Sci.* 65(3): 1005-1011.
- Gill, C. O., J. C. McGinnis, K. Rahn, D. Young, N. Lee, and S. Barbut. 2005. Microbiological condition of beef mechanically tenderized at a packing plant. *Meat Sci.* 69(4): 811-816.
- Gok, V., S. Kayaardi, and E. Obuz. 2009. Extending the chilled shelf life of vacuum-packed ground beef using ascorbic acid, nitrite or salt. *J. Muscle. Foods.* 20: 211-226.
- Goll, D., V. Thompson, H. Li, W. Wei, and J. Cong. 2003. The calpain system. *Physiol. Rev.* 83(3):731-801
- Grobbel, J. P., M. E. Dikeman, M. C. Hunt, and G. A. Milliken. 2008. Effects of different packaging atmospheres and injection-enhancement on beef tenderness, sensory attributes, desmin degradation, and display color. *J. Anim. Sci.* 86: 2697-2710.
- Gullett, E. A., S. Battenham, and T. Hore. 1996. Effect of age and cut on consistency of tenderness and leanness of beef. *Food Qual. Pref.* 7(1): 37-45.
- Hall, P. W. Jr. 1995. Chemical, physical and sensory characteristics of beef steaks processed by marination or from multineedle fluid injection of whole boneless bottom rounds. *Dissertation Abstracts International*, B. 56(2): 585
- Hamling, A. E., and C. R. Calkins. 2008. Enhancement of beef chuck and loin muscles with ammonium hydroxide and salt. *J. Anim. Sci.* 86(4): 967-971.
- Hammond, 1932. *Growth and Development of Mutton Qualities in the Sheep*. Oliver and Boyd, London. Cited from: Lawrie, R. A., and D. A. Ledward. 2006. *Lawrie's Meat Science: Seventh Edition*. CRC Press, Woodhead Publishing Limited, Cambridge, England.
- Harris, S. E., E. Huff-Lonergan, S. M. Lonergan, W. R. Jones, and D. Rankins. 2001. Antioxidant status affects color stability and tenderness of calcium chloride-injected beef. *J. Anim. Sci.* 79(3): 666-677.
- Herring, H.K., Cassens, R. G. and Briskey, E.J. 1965. Further studies on bovine muscle tenderness as influenced by carcass position, sarcomere length, and fiber diameter. *J. Food Sci.* 30, 1049-1054.
- Hildrum, K. I., B. N. Nilsen, and M. Wahlgren. 2002. Improving the tenderness of hot boned beef muscles - restriction methods of rigor mortis contraction can reduce the effects of cold shortening. *Fleischwirtschaft.* 82(5): 114-121.

- Hildrum, K. I., R. Rodbotten, M. Hoy, J. Berg, B. Narum, and J. P. Wold. 2009. Classification of different bovine muscles according to sensory characteristics and Warner Bratzler shear force. *Meat Sci.* 83(2): 302-307.
- Hilton, G.G., J.D. Tatum, S.E. Williams, K.E. Belk, F.L. Williams, J.W. Wise, and G.C. Smith. 1998. An evaluation of current and alternative systems for quality grading carcasses of mature slaughter cows. *J. Anim. Sci.* 76: 2094-2103.
- Hodgson, R.R., K.E. Belk, J.W. Savell, H.R. Cross, and F.L. Williams. 1992. Development of a quantitative quality grading system for mature cow carcasses. *J. Anim. Sci.* 70: 1840-1847.
- Hoffman, L. C. 2006. Sensory and physical characteristics of enhanced vs. non-enhanced meat from mature cows. *Meat Sci.* 72(2): 195-202.
- Hoffman, L. C., M. Muller, and A. Vermaak. 2008. Sensory and preference testing of selected beef muscles infused with a phosphate and lactate blend. *Meat Sci.* 80: 1055-1060.
- Huffman, K. L., M. F. Miller, L. C. Hoover, C. K. Wu, H. C. Brittin, and C. B. Ramsey. 1996. Effect of beef tenderness on consumer satisfaction with steaks consumed in the home and restaurant. *J. Anim. Sci.* 74: 91-97.
- Hostetler, R. L., W. A. Landmann, B. A. Link, and H. A. Fitzhugh. 1970a. Influence of carcass position during rigor mortis on tenderness of beef muscles: Comparison of two treatments. *J. Anim. Sci.* 31(1): 47-50.
- Hostetler, R. L., B. A. Link, W. A. Landmann, and H. A. Fithugh. 1972. Effect of carcass suspension on sarcomere length and shear force of some major bovine muscles. *J. Food Sci.* 37(1): 132-135.
- Hostetler, R. L., B. A. Link, W. A. Landmann, and H. A. Fithugh. 1973. Effect of carcass suspension method on sensory panel scores for some major bovine muscles. *J. Food Sci.* 38(2): 264-267.
- Hostetler, R. L., B. A. Link, W. A. Landmann, H. A. Fitzhugh, Z. L. Carpenter, and F. A. Orts. 1970b. Texas A&M tenderstretch beef; a method of carcass suspension for improving tenderness. *Tex. Agr. Exp. Sta. MP*: p.9434.
- Hui, Y. H., Nip, W., Rogers, R.W. and O. A. Young. 2001. *Meat Science and Applications*. Marcel Dekker inc., New York, NY.
- Inkelaar, P. A., and J. Fortuin. 1969. Determining the emulsifying and emulsion stabilizing capacity of protein meat additives. *Food Technol.* 23(1): 103-107.
- Ismail, H. A., E. J. Lee, K. Y. Ko, and D. U. Ahn. 2009. Fat content influences the color, lipid oxidation, and volatiles of irradiated ground beef. *J. Food Sci.* 74: 432-440.
- Janz, J. A. M., J. L. Aalhus, M. E. R. Dugan, and M. A. Price. 2006. A mapping method for the description of Warner-Bratzler shear force gradients in beef *longissimus thoracis et lumborum* and *semitendinosus*. *Meat Sci.* 72(1): 79-90.

- Janz, J. A. M., Z. Pietrasik, J. L. Aalhus, and R. J. Shand. 2005. The effects of enzyme and phosphate injections on the quality of beef *semitendinosus*. *Can. J. Anim. Sci.* 85(3): 327-334.
- Jurie, C., B. Picard, J. F. Hocquette, E. Dransfield, D. Micol, and A. Listrat. 2007. Muscle and meat quality characteristics of Holstein and Salers cull cows. *Meat Sci.* 77(4): 459-466.
- Kijowski, J., and M. G. Mast. 1993. Tenderization of spent fowl drumsticks by marination in weak organic solutions. *J. Food Technol.* 28(4): 337-342.
- Killinger, K. M., C. R. Calkins, W. J. Umberger, D. M. Feuz, and K. M. Eskridge. 2004. Consumer sensory acceptance and value for beef steaks of similar tenderness, but differing in marbling level. *J. Anim. Sci.* 82: 3294-3301.
- Kim, Y. H., Hunt M. C., Mancini, R. A., Seyfert, M., Loughin, T. M., Kropf, D. H. et al. 2006. Mechanism for lactate-color stabilization in injection-enhanced beef. *J. Agric. Food Chem.* 54(20): 7856-7862.
- Kim, Y. H., K. C. Nam, and D. U. Ahn. 2002. Volatile profiles, lipid oxidation and sensory characteristics of irradiated meat from different animal species. *Meat Sci.* 61(3): 257-265.
- Kolczak, T., K. Krzysztoforski, and K. Palka. 2008. Effect of postmortem ageing, method of heating and reheating on collagen solubility, shear force and texture parameters of bovine muscles. *Polish J. of Food and Nutri. Sci.* 58(1): 27-32.
- Koohmaraie, M., M. P. Kent, S. D. Shackelford, E. Veiseth, and T. L. Wheeler. 2002. Meat tenderness and muscle growth: Is there any relationship? *Meat Sci.* 62(3): 345-352.
- Koohmaraie, M., G. Whipple, and J. D. Crouse. 1990. Acceleration of postmortem tenderization in lamb and Brahman-cross beef carcasses through infusion of calcium-chloride. *J. Anim. Sci.* 68(5): 1278-1283.
- Kruggel, W. G., R. A. Field, and G. J. Miller. 1970. Physical and chemical properties of epimysial acid-soluble collagen from meats of varying tenderness. *J. Food Sci.* 35(2): 106-&.
- Kuehl, Robert. 2000. *Design of Experiments: Statistical Principles of Research Design and Analysis*. 2nd ed. Brooks/Cole, Belmont, CA. 81 pp.
- Lamkey, J.W., K.A. Dunlavy, and H.. Dolezal. 1993. Changes in the palatability of beef chuck muscles with the addition of sodium lactate and soy protein isolate. *J. Muscle Foods.* 4 (3): 193-206.
- Lansdell, J. L., M. F. Miller, T. L. Wheeler, M. Koohmaraie, and C. B. Ramsey. 1995. Postmortem injection of calcium-chloride effects on beef quality traits. *J. Anim. Sci.* 73(6): 1735-1740.
- Lawrence, T. E., M. E. Dikeman, M. C. Hunt, C. L. Kastner, and D. E. Johnson. 2004. Effects of enhancing beef *longissimus* with phosphate plus salt, or calcium lactate plus non-phosphate water binders plus rosemary extract. *Meat Sci.* 67(1): 129-137.

- Lawrence, T. E., M. E. Dikeman, M. C. Hunt, C. L. Kastner, and D. E. Johnson. 2003a. Effects of calcium salts on beef *longissimus* quality. *Meat Sci.* 64(3): 299-308.
- Lawrence, T. E., M. E. Dikeman, M. C. Hunt, C. L. Kastner, and D. E. Johnson. 2003b. Staged injection marination with calcium lactate, phosphate and salt may improve beef water-binding ability and palatability traits. *Meat Sci.* 65(3): 967-972.
- Lawrie, R. A., and D. A. Ledward. 2006. *Lawrie's Meat Science: Seventh Edition*. CRC Press, Woodhead Publishing Limited, Cambridge, England.
- Lennon, A. M., S. S. Moon, P. Ward, E. E. O'Neill, and T. Kenny. 2006. Effects of enhancement procedures on whole and re-formed beef forequarter muscles. *Meat Sci.* 72(3): 513-517.
- Lewis, G. J., P. P. Purslow, and A. E. Rice. 1991. The effect of conditioning on the strength of perimysial connective tissue dissected from cooked meat. *Meat Sci.* 30(1): 1-12.
- Ludwig, C. J., J. R. Claus, N. G. Marriott, J. Johnson, and H. Wang. 1997. Skeletal alteration to improve beef *longissimus* muscle tenderness. *J. Anim. Sci.* 75: 2404-2410.
- Lund, M. N., M. S. Hviid, and L. H. Skibsted. 2007. The combined effect of antioxidants and modified atmosphere packaging on protein and lipid oxidation in beef patties during chill storage. *Meat Sci.* 76(2): 226-233.
- Lynch, M. P., J. P. Kerry, D. J. Buckley, C. Faustman, and P. A. Morrissey. 1999. Effect of dietary vitamin E supplementation on the colour and lipid stability of fresh, frozen and vacuum-packaged beef. *Meat Sci.* 52(1): 95-99.
- Maca, J. V., R. K. Miller, J. D. Maca, and G. R. Acuff. 1997. Microbiological, Sensory and Chemical Characteristics of Vacuum-Packaged Cooked Beef Top Rounds Treated with Sodium Lactate and Sodium Propionate. *J. Food Sci.* 63(2): 586-590.
- Mancini, R. A., and M. C. Hunt. 2005. Current research in meat color. *Meat Sci.* 71: 100-121.
- Mancini, R. A., M. C. Hunt, K. A. Hachmeister, M. A. Seyfert, D. H. Kropf, D. E. Johnson et al. 2005. The utility of lactate and rosemary in beef enhancement solutions: effects on *longissimus* color changes during display. *J. Muscle Foods.* 16(1): 27-36.
- Mandell, I. B., C. R. Campbell, V. M. Quinton, and J. W. Wilton. 2006. Effects of skeletal separation method and postmortem ageing on carcass traits and shear force in cull cow beef. *Can. J. Anim. Sci.* 86(3): 351-361.
- Mandell, I. B., E. A. Gullett, J. Buchanan-Smith, and C. P. Campbell. 1997. Effects of diet and slaughter endpoint on carcass composition and beef quality in charolais cross steers. *Can. J. Anim. Sci.* 77(3): 403-414.
- Marsh, B. B., and N. G. Leet. 1966. Studies in meat tenderness. III. the effects of cold shortening on tenderness. *J. Food Sci.* 31(3): 450-459.

- Moeller and Courington. 1998. Branded beef study: Consumers want improved eating consistency from a branded beef product, not a pretty label. National Cattleman's Beef Association, Englewood, Colorado.
- McCormick, R. J. 1994. The flexibility of the collagen compartment of muscle. *Meat Sci.* 36: 79-91.
- McCormick, R.J., 2009. Collagen. Cited from: Du et al., 2009. *Applied Muscle Biology and Meat Science*. CRC Press, Taylor and Francis Group, LLC.
- McGregor, E. M. 2008. Effect of Nutritional Regimen and Breed on Growth Performance, Carcass Characteristics, and Meat Quality in Beef Cattle. University of Guelph, ON. CA. Thesis. 41 pp.
- McKenna, D., D. Roeber, P. Bates, T. Schmidt, D. Hale, D. Griffin et al. 2002. NBQA-2000: Survey of U.S. carcass characteristics related to quality, quantity, and value of fed steers and heifers. Rome, Italy.
- Meilgaard, M., Civille, G.V., & Carr, T. (1999). *Sensory Evaluation Techniques*, 3rd Edition. CRC Press LLC, Boca Raton, Florida, U.S.A.
- Miller, M. 2007. Dark, Firm and Dry Beef. National Cattlemen's Beef Association, on behalf of The Beef Checkoff Program. Centennial, CO, U.S.A.
- Mitsumoto, M., R. G. Cassens, D. M. Schaeffer, and K. K. Scheller. 1991. Pigment stability improvement in beef steak by ascorbic acid application. *J. Food Sci.* 56: 857-858.
- Molins, R. A., A. A. Kraft, H. W. Walker, R. E. Rust, D. G. Olson, and K. Merkenich. 1987. Effect of inorganic polyphosphates on ground-beef characteristics - some chemical, physical, and sensory effects on frozen beef patties. *J. Food Sci.* 52(1): 50-52.
- Morgan, J. B., R. K. Miller, F. M. Mendez, D. S. Hale, and J. W. Savell. 1991. Using calcium-chloride injection to improve tenderness of beef from mature cows. *J. Anim. Sci.* 69(11): 4469-4476.
- Morris, C. A., R. L. Theis, R. K. Miller, G. R. Acuff, and J. W. Savell. 1997. Improving the flavor of calcium chloride and lactic acid injected mature beef top round steaks. *Meat Sci.* 45(4): 531-537.
- Mukherjee, A., Y. Yoon, K. E. Belk, J. A. Scanga, G. C. Smith, and J. N. Sofos. 2008. Thermal inactivation of escherichia coli O157:H7 in beef treated with marination and tenderization ingredients. *J. Food Prot.* 71(7): 1349-1356.
- Nishimura, T., A. Hattori, and K. Takahashi. 1995. Structural weakening of intramuscular connective tissue during conditioning of beef. *Meat Sci.* 39(1): 127-133.
- Nishimura, T., A., Hattori, and K. Takahashi. 1996. Relationship between degradation of proteoglycans and weakening of the intramuscular connective tissue during postmortem ageing of beef. *Journal of Meat Science.* 42: 251-260. Cited from: Du et al., 2009. *Applied Muscle Biology and Meat Science*. CRC Press, Taylor and Francis Group, LLC.

- Novakofski, J., and S. Brewer. 2006. The paradox of toughening during the aging of tender steaks. *J. Food Sci.* 71(6): 473-479.
- NPPC, 2000. Pork Composition & Quality Assessment Procedures. National Pork Producers Council, Des Moines, Iowa.
- Offer, G., D. Restall, and J. Trinick. 1984. Water-Holding in Meat. Recent advances in the chemistry of meat / proceedings of a symposium, ARC Meat Research Institute, Langford, Bristol, U.K.
- Offer, G., and J. Trinick. 1983. On the mechanism of water holding in meat: The swelling and shrinking of myofibrils. *Meat Sci.* 8(4): 245-281.
- Okayama, T., T. Imai, and M. Yamanoue. 1987. Effect of ascorbic acid and alpha-tocopherol on storage stability of beef steaks. *Meat Science.* 21: 267-273.
- Onenc, A., M. Serdaroglu, and K. Abdraimov. 2004. Effect of various additives to marinating baths on some properties of cattle meat. *Eur. Food Res. Technol.* 218: 114-117.
- Oury, M. P., B. Picard, M. Briand, J. P. Blanquet, and R. Dumont. 2009. Interrelationships between meat quality traits, texture measurements and physicochemical characteristics of M. rectus abdominis from Charolais heifers [electronic resource]. *Meat Sci.* 83(2): 293-301.
- Page, J. K., D. M. Wulf, and T. R. Schwotzer. 2001. A survey of beef muscle color and pH. *J. Anim. Sci.* 79(3): 678-687.
- Papadopoulos, L. S., R. K. Miller, G. R. Acuff, C. Vanderzant, and H. R. Cross. 1991a. Effect of sodium lactate on microbial and chemical-composition of cooked beef during storage. *J. Food Sci.* 56(2): 341-347.
- Papadopoulos, L. S., R. K. Miller, L. J. Ringer, and H. R. Cross. 1991b. Sodium lactate effect on sensory characteristics, cooked meat color and chemical-composition. *J. Food Sci.* 56(3): 621-&.
- Papadopoulos, L. S., R. K. Miller, G. R. Acuff, L. M. Lucia, C. Vanderzant, and H. R. Cross. 1991c. Consumer and trained sensory comparisons of cooked beef top rounds treated with sodium lactate. *J. Food Sci.* 56(5): 1141-1146.
- Penny, I. F. 1977. The effect of temperature on the drip, denaturation and extracellular space of pork *longissimus dorsi* muscle. *J. Sci. Food Agric.* 28(4): 329-338.
- Pietrasik, Z., J. L. Aalhus, L. L. Gibson, and P. J. Shand. 2010. Influence of blade tenderization, moisture enhancement and pancreatin enzyme treatment on the processing characteristics and tenderness of beef *semitendinosus* muscle. *Meat Sci.* : 512-517.
- Platter, W. J., J. D. Tatum, K. E. Belk, S. R. Koontz, P. L. Chapman, and G. C. Smith. 2005. Effects of marbling and shear force on consumers' willingness to pay for beef strip loin steaks. *J. Anim. Sci.* 83(4): 890-899.
- Poste, L.M., Mackie, D.A., Butler, G. and Larmond, E. 1991. Laboratory Methods for Sensory Analysis of Food. Publication #1637. Canada Department of Agriculture.

- Powell, V. H. 1991. Quality of beef loin steaks as influenced by animal age, electrical stimulation and ageing. *Meat Sci.* 30(3): 195-205.
- Purchas, R. W. 1990. An assessment of the role of pH differences in determining the relative tenderness of meat from bulls and steers. *Meat Sci.* 27(2): 129-140.
- Purchas, R. W., and R. Aungsupakorn. 1993. Further investigations into the relationship between ultimate pH and tenderness for beef samples from bulls and steers. *Meat Sci.* 34(2): 163-178.
- Raharjo, S., J. N. Sofos, and G. R. Schmidt. 1993. Solid-phase acid extraction improves thiobarbituric acid method to determine lipid oxidation. *J. Food Sci.* 58(4): 921-924.
- Ramsbottom, J.M., and E.J. Strandine. 1948. Comparative tenderness and identification of muscles in wholesale beef cuts. *J. Food Res.* 13: 315.
- Rees, M. P., G. R. Trout, and R. D. Warner. 2002. Effect of calcium infusion on tenderness and ageing rate of pork m. *longissimus* thoracis et lumborum after accelerated boning. *Meat Sci.* 61(2): 169-179.
- Renerre, M. 1990. Factors involved in the discoloration of beef meat. *Int. J. Food Sci. Tech.* 25: 613-630.
- Robbins, K., J. Jensen, K.J. Ryan, C. Homco-Ryan, F.K. McKeith, and M.S. Brewer. 2003. Consumer attitudes towards beef and acceptability of enhanced beef. *Meat Sci.* 65: 721-729.
- Rodas-González, A., N. Huerta-Leidenz, N. Jerez-Timaure, and M. F. Miller. 2009. Establishing tenderness thresholds of Venezuelan beef steaks using consumer and trained sensory panels. *Meat Sci.* : 218-223.
- Rodbotten, R. and K.I. Hildrum. 2000. Sarcomere length as indicator of tenderness in bovine *M. longissimus* dorsi exposed to different post mortem treatments. Proceedings of the 46th International Congress of Meat Science and Technology Buenos Aires, Argentina. pp.480-481.
- Rosenvold, K., M. North, C. Devine, E. Micklander, P. Hansen, P. Dobbie et al. 2008. The protective effect of electrical stimulation and wrapping on beef tenderness at high pre rigor temperatures. *Meat Sci.* 79(2): 299-306.
- Saffle, R.L. and Bratzler, L.J. 1959. The effect of fatness on some processing and palatability characteristics of pork carcasses. *Food Technol.* 13:236-239.
- Sanchez-Escalante, A., D. Djenane, G. Torrecano, J. A. Beltran, and P. Roncales. 2001. The effects of ascorbic acid, taurine, carnosine and rosemary powder on colour and lipid stability of beef patties packaged in modified atmosphere. *Meat Sci.* 58(4): 421-429.
- SAS Institute Inc., (2007). SAS Version 9.2, 2007. SAS Institute, Inc., Cary, N.C.
- Sawyer, J. E., C. P. Mathis, and B. Davis. 2004. Effects of feeding strategy and age on live animal performance, carcass characteristics, and economics of short-term feeding programs for culled beef cows. *J. Anim. Sci.* 82(12): 3646-3653.
- Schnell, T. D., K. E. Belk, J. D. Tatum, R. K. Miller, and G. C. Smith. 1997.

- Performance, carcass, and palatability traits for cull cows fed high-energy concentrate diets for 0, 14, 28, 42, or 56 days. *J. Anim. Sci.* 75: 1195-1202.
- Schreurs N. M., F. Garcia, C. Jurie, J. Agabriel, D. Micol, and D. Bauchar. 2008. Meta-analysis of the effect of animal maturity on muscle characteristics in different muscles, breeds, and sexes of cattle. *J. Anim. Sci.* 86(11): 2872-2887.
- Schroeder, T.C. and D.R. Mark. 2000. How can the beef industry recapture lost consumer demand?. *J. Anim. Sci.* 2000. 77: 1-13.
- Serdaroglu, M., K. Abdraimov, and A. Onenc. 2007. The effects of marinating with citric acid solutions and grapefruit juice on cooking and eating quality of turkey breast. *J. Muscle Foods.* 18: 162-172.
- Seyfert, M., M. C. Hunt, R. A. Mancini, K. A. Hachmeister, D. H. Kropf, and J. A. Unruh. 2004. Accelerated chilling and modified atmosphere packaging affect colour and colour stability of injection-enhanced beef round muscles. *Meat Sci.* 68(2): 209-219
- Seyfert, M., M. C. Hunt, M. L. Ahnstrom, and D. E. Johnson. 2007. Efficacy of lactic acid salts and sodium acetate on ground beef colour stability and metmyoglobin-reducing activity. *Meat Sci.* 75(1): 134-142.
- Seyfert, M., R. A. Mancini, M. C. Hunt, J. L. Tang, C. Faustman, and M. Garcia. 2006. Color stability, reducing activity, and cytochrome c oxidase activity of five bovine muscles. *J. Agric. Food Chem.* 54(23): 8919-8925.
- Shackelford, S. D., M. Koohmaraie, L. V. Cundiff, K. E. Gregory, G. A. Rohrer, and J. W. Savell. 1994. Heritabilities and phenotypic and genetic correlations for bovine postrigor calpastatin activity, intramuscular fat content, Warner-Bratzler shear force, retail product yield, and growth rate. *J. Anim. Sci.* 72: 857-863.
- Shackelford, S. D., M. Koohmaraie, G. Whipple, T. L. Wheeler, M. F. Miller, J. D. Crouse et al. 1991. Predictors of beef tenderness - development and verification. *J. Food Sci.* 56(5): 1130-&.
- Shanks, B. C., D. M. Wulf, B. J. Reuter, and R. J. Maddock. 2002. Increasing tenderness of beef round and sirloin muscles through prerigor skeletal separations. *J. Anim. Sci.* 80(1): 123-128.
- Shemeis, A. R., T. Liboriussen, B. B. Andersen, and O. Y. Abdallah. 1994. Changes in carcass and meat quality traits of danish friesland cull cows with the increase of their age and body condition. *Meat Sci.* 37(2): 161-167.
- Shen, Q.W., Du, M., Means, W.J. Regulation of Postmortem Glycolysis and Meat Quality. Cited from: *Applied Muscle Biology and Meat Science*. CRC Press, Taylor and Francis Group, LLC.
- Smith, L. A., S. L. Simmons, F. K. McKeith, P. J. Bechtel, and P. L. Brady. 1984. Effects of sodium tripolyphosphate on physical and sensory properties of beef and pork roasts. *J. Food Sci.* 49(6): 1636-&.
- Soerheim, O., and K. I. Hildrum. 2002. Muscle stretching techniques for improving meat tenderness. *Trends Food Sci. Technol.* 13(4): 127-135.

- Soerheim, O., J. Idland, E. C. Halvorsen, T. Froystein, P. Lea, and K. I. Hildrum. 2001. Influence of beef carcass stretching and chilling rate on tenderness of *m. longissimus dorsi*. *Meat Sci.* 57(1): 79-85.
- Spanier, A. M., Edwards, J. F. and Dupuy, H. P. 1988. The warmed-over flavor process in beef. A study of meat proteins and peptides. *J. Food Tech.* 42(6):110-118.
- Squires, E. J., E. V. Valdes, J. Wu, and S. Leeson. 1991. Utility of the thiobarbituric acid test in the determination of the quality of fats and oils in feeds. *Poult. Sci.* 70(1): 180-183.
- Statistics Canada, 2009. Livestock Statistics: Cull Cows on Farm, January 1, 2009.
- Statistics Canada, 2010. Livestock Statistics: Catalogue no. 23-012-X.
- St. Angelo, A. J., Crippen, K. L., Dupuy, H. P., and James, C. Jr. 1990. Chemical and sensory studies of antioxidant treated beef. *J. Food Sci.* 55:1501-1505, 1539.
- Stelzl, A. M., L. E. Patten, D. D. Johnson, C. R. Calkins, and B. L. Gwartney. 2007. Benchmarking carcass characteristics and muscles from commercially identified beef and dairy cull cow carcasses for Warner-Bratzler shear force and sensory attributes. *J. Anim. Sci.* 85: 2631-2638.
- Stivarius, M. R., F. W. Pohlman, K. S. McElyea, and J. K. Apple. 2002a. The effects of acetic acid, gluconic acid and trisodium citrate treatment of beef trimmings on microbial, color and odor characteristics of ground beef through simulated retail display. *Meat Sci.* 60(3): 245-252.
- Stivarius, M. R., F. W. Pohlman, K. S. McElyea, and J. K. Apple. 2002b. Microbial, instrumental color and sensory color and odor characteristics of ground beef produced from beef trimmings treated with ozone or chlorine dioxide. *Meat Sci.* 60(3): 299-305.
- Stolowski, G. D., B. E. Baird, R. K. Miller, J. W. Savell, A. R. Sams, J. F. Taylor et al. 2006. Factors influencing the variation in tenderness of seven major beef muscles from three Angus and Brahman breed crosses. *Meat Sci.* 73(3): 475-483.
- Stone, H., and J. L. Sidel. 2004. Sensory evaluation practices. Amsterdam ; Boston : Elsevier Academic Press.
- Takahashi K. 1996. Structural weakening of skeletal muscle tissue during postmortem ageing of meat: The non-enzymatic mechanism of meat tenderization. *Meat Sci.* 43(Suppl.): 67-80.
- Takahashi, K., A. Hattori, and H. Kuroyanagi. 1995. Relationship between the translocation of paratropomyosin and the restoration of rigor-shortened sarcomeres during postmortem aging of meat - a molecular mechanism of meat tenderization. *Meat Sci.* 40(3): 413-423.
- Thomson, B. C., and P. M. Dobbie. 1997. The effect of calcium chloride treatment on shear force and weight loss in gluteus medius and *longissimus* muscles from pasture fed bulls. *N. Z. J. Agric. Res.* 40(4): 507-512.

- Torrescano, G., A. Sanchez-Escalante, B. Gimenez, P. Roncales, and J. A. Beltran. 2003. Shear values of raw samples of 14 bovine muscles and their relation to muscle collagen characteristics. *Meat Sci.* 64(1): 85-91.
- USDA. 2005. Livestock slaughter-2004 summary. USDA, Natl. Agric. Stat. Serv., Washington, DC.
- Varnam, A. H., and J. P. Sutherland. 1995. *Meat and Meat Products :Technology, Chemistry and Microbiology*. Chapman & Hall, London, England.
- Vote, D. J., W. J. Platter, J. D. Tatum, G. R. Schmidt, K. E. Belk, G. C. Smith et al. 2000. Injection of beef strip loins with solutions containing sodium tripolyphosphate, sodium lactate, and sodium chloride to enhance palatability. *J. Anim. Sci.* 78(4): 952-957.
- Wang, H., J. R. Claus, and N. G. Marriott. 1994. Selected skeletal alterations to improve tenderness of beef round muscles. *J. Muscle Foods.* 5(2): 137-147.
- Wheeler, T. L., M. Koohmaraie, and J. D. Crouse. 1991. Effects of calcium-chloride injection and hot boning on the tenderness of round muscles. *J. Anim. Sci.* 69(12): 4871-4875.
- Wheeler, T. L., M. Koohmaraie, J. L. Lansdell, G. R. Siragusa, and M. F. Miller. 1993. Effects of postmortem injection time, injection level, and concentration of calcium-chloride on beef quality traits. *J. Anim. Sci.* 71(11): 2965-2974.
- Wheeler, T. L., M. Koohmaraie, and S. D. Shackelford. 1996. Effect of vitamin C concentration and co-injection with calcium chloride on beef retail display color. *J. Anim. Sci.* 74(8): 1846-1853.
- Wheeler, T. L., S. D. Shackelford, I. P. Johnson, M. F. Miller, R. K. Miller, and M. Koohmaraie. 1997a. A comparison of Warner-Bratzler shear force assessment within and among institutions. *J. Anim. Sci.* 75(9): 2423-2432.
- Wheeler, T. L., M. Koohmaraie, and S. D. Shackelford. 1997b. Effect of postmortem injection time and postinjection aging time on the calcium-activated tenderization process in beef. *J. Anim. Sci.* 75(10): 2652-2660.
- Whipple, G., and M. Koohmaraie. 1993. Calcium-chloride marination effects on beef steak tenderness and calpain proteolytic activity. *Meat Sci.* 33(2): 265-275.
- Witte, V. C., G. F. Krause, and M. E. Bailey. 1970. A new extraction method for determining 2 thio barbituric-acid values of pork and beef during storage. *J. Food Sci.* 35(5): 582-585.
- Wu, J., and E. J. Squires. The effect of dietary corn oil, vitamin E, and selenium on lipid peroxidation and hemorrhage in chicken liver. *J. Nutr. Biochem.* 8(11): 629-633.
- Wulf, D. M., R. S. Emmett, J. M. Leheska, and S. J. Moeller. 2002. Relationships among glycolytic potential, dark cutting (dark, firm, and dry) beef, and cooked beef palatability. *J. Anim. Sci.* 80(7): 1895-1903.

Xiong Y. L., Mullins O. E., Stika J. F., Chen J., Blanchard S. P., and Moody W. G. 2007. Tenderness and oxidative stability of post-mortem muscles from mature cows of various ages. *Meat Sci.* 77(1): 105-113.

Appendix Table 1: Overall Analysis of Variance for Carcass Characteristics for Cull Cow Beef									
Trait	Date of Slaughter	Side Weight	Side	Slaughter Age	Grade Fat	Postmortem Processing Treatment ^y	Breed ^t	Postmortem Processing Treatment* Breed	
Age at Slaughter, (yrs)	0.606	0.403	0.945	-	0.299	0.571	0.590	0.470	
Hot Carcass Wt, (kg)	0.254	<0.001	0.703	0.295	0.603	0.115	0.344	0.093	
Side Carcass Wt, (kg)	0.298	-	0.667	0.403	0.831	0.129	0.348	0.112	
Backfat at ¼ position ^z , (mm)	0.038	<0.001	0.264	0.758	-	0.920	0.127	0.852	
Backfat at ½ position ^y	0.167	<0.001	0.087	0.608	-	0.797	0.006	0.848	
Backfat at ¾ position ^x	0.567	<0.001	0.208	0.515	-	0.921	0.031	0.950	
Grade Fat ^w , (mm)	0.589	0.831	0.309	0.299	-	0.881	0.032	0.870	
Marbling Score ^v	0.697	0.070	0.296	0.021	<0.001	0.423	0.045	0.878	
LMA ^u , cm ²	0.534	0.705	0.839	0.014	0.539	0.819	<0.001	0.904	

^z Backfat at ¼ position = mm of grade fat in the first quadrant over the LM.
^y Backfat at ½ position = mm of grade fat in the second quadrant over the LM.
^x Backfat at ¾ position = mm of grade fat in the third quadrant over the LM.
^w Grade fat = mm of grade fat in the last quadrant over the LM.
^v Marbling Score – (0 = Devoid; 1 =Grade A; 2 =Grade AA; 3 =Grade AAA; 4=Prime).
^u *Longissimus* Muscle Area.
^t Breed designates cattle with predominantly British or Continental breeding.

Appendix Table 2: Overall Analysis of Variance for Carcass Drop Measurements^z

Trait	Date of Slaughter	Side	Side Weight	Slaughter Age	Grade Fat	Postmortem Processing Treatment ^y	Breed	Postmortem Processing Treatment* Breed
Pre-Skeletal Separation Carcass Length (cm)	<0.001	0.065	<0.001	0/079	0.002	0.162	0.944	0.626
Post-Skeletal Separation Carcass Length (cm)	<0.001	0.165	<0.001	0.673	0.008	<0.001	0.860	0.993
Carcass Drop 1 h Postmortem (cm)	0.108	0.890	0.763	0.091	0.564	<0.001	0.744	0.596
Carcass Drop 24 h Postmortem (cm)	0.768	0.272	0.422	0.049	0.781	<0.001	0.888	0.209

^z -Measurements of Pre- and Post-Skeletal Separation occurred within 1 h of slaughter. Carcass Drop measurements were taken at indicated time. Statistical model includes the following; Date of slaughter; Side = left or right carcass side; Side weight in kg; Age at slaughter in years; Grade fat = mm of grade fat in the last quadrant over the LM; Postmortem Processing Treatment which is described below; Breed= British or Continental; Postmortem Processing Treatment by Breed Interaction.

^y -Treatment= Either No Postmortem Processing (NPP), Skeletal Separation (SS), Moisture Enhancement (ME), or Skeletal Separation and Moisture Enhancement (SS/ME).

Appendix Table 3: Overall Analysis of Variance for Traits Involving the <i>Longissimus</i> (Loin) on Initial day of Processing, After 14 days of Ageing, and After Evaluation of Shelf Life ^z						
Trait	Slaughter Age	Grade Fat	Side	Breed ^u	Treatment ^t	Treatment*breed
Initial Day of Processing						
pH	0.186	0.468	0.722	0.627	0.572	0.102
L*	0.002	0.020	0.158	0.277	0.176	0.217
a	0.336	0.055	0.820	0.120	0.001	0.514
b	0.828	0.202	0.102	0.143	0.039	0.065
Hue ^w	0.560	0.285	0.106	0.187	0.102	0.054
Chroma ^v	0.404	0.040	0.989	0.110	<0.001	0.423
Sarcomere Length, μ m	0.347	0.487	0.502	0.948	<0.001	0.407
14 Day Aged Product						
pH	0.227	0.561	0.522	0.816	0.030	0.552
L*	0.088	0.030	0.856	0.873	0.001	0.624
a	0.745	0.291	0.934	0.117	0.002	0.114
b	0.457	0.016	0.221	0.684	0.570	0.013
Hue	0.422	0.017	0.173	0.990	0.078	0.016
Chroma	0.940	0.138	0.719	0.143	0.014	0.041
TBARS ^y , mg MDA/kg ^x	0.850	0.204	0.126	0.438	0.470	0.504
Shelf Life						
pH	0.042	0.504	0.151	0.700	0.074	0.886
L*	0.046	<0.001	0.576	0.512	0.076	0.379
a	0.025	0.005	0.249	0.305	<0.001	0.147
b	<0.001	<0.001	0.051	0.749	0.668	0.418
Hue	0.007	<0.001	0.146	0.472	0.314	0.589
Chroma	0.007	<0.001	0.168	0.384	<0.001	0.122
Drip loss, %	0.5778	0.400	0.053	0.066	<0.001	0.003
^z Initial Day of Processing refers to samples taken after Moisture Enhancement where appropriate (approximately 24 h postmortem for all muscles); 14 Day Aged Product refers to steaks that have been aged at < 4°C until 14 d post mortem; Shelf Life refers to measurements on 18 d aged steaks where 14 d aged steaks are placed on foam trays and overwrapped with PVC film and stored at < 4°C in a chill cooler for an additional 4 d before sampling. ^y TBARS = Thiobarbituric Acid Reactive Substances; ^x MDA = Malonaldehyde; ^w Hue = $\tan^{-1}(b^*/a^*)$; ^v Chroma = Square root of $((a^*)^2 + (b^*)^2)$. ^u Breed represents either predominantly British (BRIT) or predominantly Continental (CONT) animals. ^t Treatment= Either No Postmortem Processing (NPP), Skeletal Separation (SS), Moisture Enhancement (ME), or Skeletal Separation and Moisture Enhancement (SS/ME).						

Appendix Table 4: Overall Analysis of Variance for Traits Involving the <i>Longissimus</i> (Ribeye) on Initial day of Processing, After 14 days of Ageing, and After Evaluation of Shelf Life ^z							
Trait	Slaughter Age	Grade Fat	Side	Breed ^u	Treatment ^t	Treatment*breed	
Initial Day of Processing							
pH	0.089	0.282	0.563	0.765	0.080	0.106	
L*	0.637	0.007	0.598	0.015	0.056	0.083	
a	0.939	0.108	0.526	0.551	<0.001	0.240	
b	0.524	0.053	0.391	0.538	0.024	0.468	
Hue ^w	0.485	0.054	0.483	0.647	0.257	0.440	
Chroma ^v	0.817	0.088	0.485	0.562	<0.001	0.256	
Sarcomere Length, μ m	0.318	0.796	0.251	0.745	<0.001	0.943	
14 Day Aged Product							
pH	0.540	0.701	0.989	0.667	0.050	0.014	
L*	0.159	0.006	0.381	0.624	<0.001	0.031	
a	0.691	0.014	0.598	0.152	0.014	0.927	
b	0.610	<0.001	0.123	0.685	0.007	0.157	
Hue	0.458	<0.001	0.090	0.932	<0.001	0.091	
Chroma	0.801	0.005	0.450	0.196	0.106	0.840	
TBARS ^y , mg MDA/kg ^x	0.011	0.411	0.261	0.753	0.005	0.536	
Shelf Life							
pH	0.084	0.741	0.407	0.332	0.487	0.978	
L*	0.055	0.001	0.236	0.769	0.190	0.784	
a	0.006	<0.001	0.152	0.077	<0.001	0.401	
b	0.002	<0.001	0.615	0.141	<0.001	0.788	
Hue	0.013	<0.001	0.841	0.393	0.173	0.703	
Chroma	0.002	<0.001	0.188	0.057	<0.001	0.449	
Drip loss, %	0.170	0.036	0.362	0.684	<0.001	0.103	
Aerobic Plate Count, cfu/cm	0.886	0.492	0.938	0.261	0.430	0.392	
^z Initial Day of Processing refers to samples taken after Moisture Enhancement where appropriate (approximately 24 h postmortem for all muscles); 14 Day Aged Product refers to steaks that have been aged at < 4°C until 14 d post mortem; Shelf Life refers to measurements on 18 d aged steaks where 14 d aged steaks are placed on foam trays and overwrapped with PVC film and stored at < 4°C in a chill cooler for an additional 4 d before sampling. ^y TBARS = Thiobarbituric Acid Reactive Substances; ^x MDA = Malonaldehyde; ^w Hue = Tan ⁻¹ (b*/a*); ^v Chroma = Square root of ((a* ²) + (b* ²)). ^u Breed represents either predominantly British (BRIT) or predominantly Continental (CONT) animals. ^t Treatment= Either No Postmortem Processing (NPP), Skeletal Separation (SS), Moisture Enhancement (ME), or Skeletal Separation and Moisture Enhancement (SS/ME).							

Appendix Table 5: Overall Analysis of Variance for Traits Involving the <i>Semimembranosus</i> (SM) on Initial day of Processing, After 14 days of Ageing, and After Evaluation of Shelf Life ^z							
Trait	Slaughter Age	Grade Fat	Side	Breed ^u	Treatment ^t	Treatment*breed	
Initial Day of Processing							
pH	0.080	0.666	0.368	0.666	0.750	0.955	
L*	0.485	0.005	0.524	0.693	0.651	0.262	
a	0.789	0.444	0.871	0.988	<0.001	0.608	
b	0.282	0.381	0.546	0.886	0.540	0.709	
Hue ^w	0.179	0.472	0.581	0.980	0.779	0.523	
Chroma ^v	0.683	0.382	0.990	0.948	0.002	0.615	
Sarcomere Length, μm	0.433	0.999	0.038	0.995	0.892	0.969	
14 Day Aged Product							
pH	0.271	0.735	0.218	0.709	0.479	0.933	
L*	0.280	0.401	0.081	0.576	0.065	0.325	
a	0.306	0.134	0.055	0.068	0.055	0.532	
b	0.312	0.030	0.003	0.915	0.971	0.866	
Hue	0.436	0.104	0.003	0.703	0.809	0.519	
Chroma	0.213	0.007	0.019	0.114	0.174	0.738	
TBARS ^y , mg MDA/kg ^x	0.050	0.601	0.537	0.003	0.031	0.219	
Shelf Life							
pH	0.023	0.055	0.359	0.292	<0.001	0.077	
L*	0.235	0.079	0.438	0.935	0.719	0.814	
a	0.042	0.056	0.053	0.179	<0.001	0.326	
b	0.2556	0.009	0.392	0.320	0.056	0.096	
Hue	0.205	0.005	0.355	0.458	0.028	0.135	
Chroma	0.121	0.007	0.217	0.633	0.155	0.371	
Drip loss, %	0.735	0.206	0.939	0.174	0.001	0.610	
^z Initial Day of Processing refers to samples taken after Moisture Enhancement where appropriate (approximately 24 h postmortem for all muscles); 14 Day Aged Product refers to steaks that have been aged at < 4°C until 14 d post mortem; Shelf Life refers to measurements on 18 d aged steaks where 14 d aged steaks are placed on foam trays and overwrapped with PVC film and stored at < 4°C in a chill cooler for an additional 4 d before sampling.							
^y TBARS = Thiobarbituric Acid Reactive Substances; ^x MDA = Malonaldehyde; ^w Hue = $\text{Tan}^{-1}(\text{b}^*/\text{a}^*)$; ^v Chroma = Square root of $((\text{a}^{*2}) + (\text{b}^{*2}))$.							
^u Breed represents either predominantly British (BRIT) or predominantly Continental (CONT) animals.							
^t Treatment = Either No Postmortem Processing (NPP), Skeletal Separation (SS), Moisture Enhancement (ME), or Skeletal Separation and Moisture Enhancement (SS/ME).							

Appendix Table 6: Overall Analysis of Variance for Traits Involving the <i>Semitenidinosus</i> (ST) on Initial day of Processing, After 14 days of Ageing, and After Evaluation of Shelf Life ^z							
Trait	Slaughter Age	Grade Fat	Side	Breed ^u	Treatment ^t	Treatment*breed	
Initial Day of Processing							
pH	0.027	0.872	0.001	0.258	0.445	0.415	
L*	0.096	0.044	0.197	0.636	0.994	0.513	
a	0.337	0.020	0.909	0.596	<0.001	0.620	
b	0.585	0.227	0.659	0.171	0.192	0.899	
Hue ^w	0.697	0.343	0.734	0.154	0.950	0.713	
Chroma ^v	0.347	0.018	0.983	0.437	<0.001	0.657	
Sarcomere Length, μ m	0.448	0.432	0.180	0.668	0.152	0.667	
14 Day Aged Product							
pH	0.618	0.147	0.936	0.297	0.040	0.949	
L*	0.115	0.155	0.376	0.455	0.032	0.742	
a	0.170	0.389	0.419	0.081	0.009	0.350	
b	0.605	0.006	0.731	0.098	0.071	0.925	
Hue	0.405	0.009	0.388	0.272	0.167	0.981	
Chroma	0.310	0.133	0.512	0.048	0.008	0.371	
TBARS ^y , mg MDA/kg ^x	0.001	0.709	0.739	0.048	0.086	0.897	
Shelf Life							
pH	0.153	0.794	0.150	0.225	0.627	0.828	
L*	0.033	0.050	0.354	0.450	0.008	0.350	
a	0.164	0.030	0.046	0.229	0.099	0.509	
b	0.065	0.065	0.093	0.432	0.104	0.193	
Hue	0.144	0.180	0.643	0.724	0.003	0.393	
Chroma	0.082	0.017	0.041	0.194	0.153	0.419	
Drip loss, %	0.511	0.602	0.486	0.204	0.001	0.075	
Aerobic Plate Count, cfu/cm	0.362	0.033	0.636	0.077	0.603	0.542	
^z Initial Day of Processing refers to samples taken after Moisture Enhancement where appropriate (approximately 24 h postmortem for all muscles); 14 Day Aged Product refers to steaks that have been aged at < 4°C until 14 d post mortem; Shelf Life refers to measurements on 18 d aged steaks where 14 d aged steaks are placed on foam trays and overwrapped with PVC film and stored at < 4°C in a chill cooler for an additional 4 d before sampling.							
^y TBARS = Thiobarbituric Acid Reactive Substances; ^x MDA = Malonaldehyde; ^w Hue = $\tan^{-1} (b^*/a^*)$; ^v Chroma = Square root of $((a^*)^2 + (b^*)^2)$							
^u Breed represents either predominantly British (BRIT) or predominantly Continental (CONT) animals.							
^t Treatment= Either No Postmortem Processing (NPP), Skeletal Separation (SS), Moisture Enhancement (ME), or Skeletal Separation and Moisture Enhancement (SS/ME).							

Appendix Table 7: Overall Analysis of Variance for Traits Involving the <i>Longissimus</i> Muscle (LM) from the Ribeye, LM from the Loin, the <i>Semimembranosus</i> Muscle (SM), and the <i>Semitendinosus</i> (ST) Muscle										
Trait	Grade fat	Slage	DOSL	Side	Trt ²	Breed	Doage	Trt*Breed	Breed* Doage	Trt*Breed *Doage
LONGISSMUS - LOIN										
Shear Force, Kg	0.181	0.168	0.094	0.211	<0.001	0.166	<0.001	0.551	0.996	0.598
Cookloss, %	0.019	0.619	<0.001	0.871	<0.001	0.904	0.008	0.088	0.783	0.799
LONGISSMUS - RIBEYE										
Shear Force, Kg	0.902	0.196	0.014	0.385	<0.001	0.426	<0.001	0.3615	0.963	0.215
Cookloss, %	0.514	0.152	<0.001	0.497	0.008	0.713	0.009	0.866	0.806	0.503
SEMIMEMBRANOSUS (SM)										
Shear Force, Kg	0.569	0.186	0.001	0.689	<0.001	0.7696	0.118	0.624	0.917	0.424
Cookloss, %	0.717	0.163	<0.001	0.509	0.281	0.619	0.020	0.673	0.198	0.384
SEMITENDINOSUS (ST)										
Shear Force, Kg	0.546	0.085	<0.001	0.306	0.029	0.033	<0.001	0.207	0.753	0.474
Cookloss, %	0.004	0.960	<0.001	0.119	0.052	0.401	0.010	0.386	0.741	0.881
Statistical model includes the following= Date of slaughter (DOSL); Side = left or right carcass side; Side weight in kg; Age at slaughter in years; Grade fat = mm of grade fat in the last quadrant over the LM; Postmortem Processing Treatment which is described below; Breed= British or Continental; Postmortem Processing Treatment by Breed Interaction.										
z. Treatment= Either No Postmortem Processing (NPP), Skeletal Separation (SS), Moisture Enhancement (ME), or Skeletal Separation and Moisture Enhancement (SS/ME).										

Appendix Table 8: Overall Analysis of Variance for Traits Involving the <i>Longissimus</i> Muscle (LM) from the Ribeye Obtained from Sensory Evaluation		
Traits	Sensory Evaluation Day	Treatment ^z by Breed ^y Interaction
Softness	<0.001	0.099
Tenderness	<0.001	0.253
Chewiness	<0.001	0.101
Rate of Breakdown	<0.001	0.118
Initial Juiciness	<0.001	<0.001
Overall Juiciness	<0.001	<0.001
Flavour	0.049	0.009
Off-flavour	0.042	0.129
Statistical model includes the following= Sensory Evaluation Day; Treatment by Breed Interaction.		
^z -Treatment= Either No Postmortem Processing (NPP), Skeletal Separation (SS), Moisture Enhancement (ME), or Skeletal Separation and Moisture Enhancement (SS/ME).		
^y Breed represents either predominantly British (BRIT) or predominantly Continental (CONT) animals.		

Appendix Table 9: Overall Analysis of Variance for Traits Involving the <i>Semitenndinosus</i> Muscle (ST) Obtained from Sensory Evaluation		
Traits	Sensory Evaluation Day	Treatment ^z by Breed ^y Interaction
Softness	<0.001	0.437
Tenderness	<0.001	0.627
Chewiness	<0.001	0.397
Rate of Breakdown	<0.001	0.510
Initial Juiciness	0.041	0.320
Overall Juiciness	0.023	0.235
Flavour	<0.001	0.043
Off-flavour	0.006	0.639
Statistical model includes the following= Sensory Evaluation Day; Treatment by Breed Interaction.		
^z -Treatment= Either No Postmortem Processing (NPP), Skeletal Separation (SS), Moisture Enhancement (ME), or Skeletal Separation and Moisture Enhancement (SS/ME).		
^y Breed represents either predominantly British (BRIT) or predominantly Continental (CONT) animals.		