University of Alberta

Implications of Canada Grade and Early Post-Mortem Carcass Management on Beef Quality

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ABSTRACT

Meat quality characteristics of *m. longissimus thoracis* (LT) from Canada A, AA, AAA and Prime grades were determined at day 3 and 14. Canada Prime was substantiated as the superior product, having the highest L*, a*, b* values and the greatest tenderness. LT steaks from Canada A & AA carcasses had the lowest crude fat content but the highest moisture and protein content. These results supported LT meat from Canada Prime beef carcasses being of the highest economic value.

The effects of early *post mortem* chiller temperature, electrical stimulation (ES) and ageing on beef quality were also studied. A significant ES/AGEING interaction indicated that ES steaks at 3 days aged had lower shear force values than those of non-ES, but this difference disappeared at 14 days. After 14 days ageing, steaks had increased L*, a* and b* values. ES improved meat appearance and tenderness but this improvement declined with time *post mortem*, indicating that ES can be used to modify time to market for beef LT steaks.

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LIST OF ABBREVIATIONS

Å	Angstrom
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
AAFC	Agriculture and Agri-Food Canada
AMSA	American Meat Science Association
AOAC	Association of Official Analytical Chemists
*b**	Blue–yellow colour coordinates/space
CIE	Commission International De I' Eclairage
CP	Creatine phosphate
DFD	Dark, firm, and dry
ES	Electrical stimulation
EDTA	Ethylenediaminetetraacetic acid
Fe^{2+}	Ferrous state
pHu	Final or ultimate pH
*a**	Green-red colour coordinates/space
Hsp	Heat shock proteins
HGP	Hormone growth promotants
P_i	Inorganic phosphate
*L**	Lightness
LL	*Longissimus lumborum*

LT	*Longissimus thoracis*
MSA	Meat Standards Australia
NIR	Near-infra-red
N	Newton
Non-ES	Non-electrically stimulated
OCT	Optimum cutting temperature
O_2	Oxygen
Pr > F	Probability of the calculated F value
SEM	Standard error of the mean
SAS	Statistical Analysis System
MTT	Tetrazolium salt
USDA	United States Department of Agriculture
VIS	Visible
WBSF	Warner-Bratzler shear force
WHC	Water holding capacity

Chapter 1

1.0 Literature review

1.1 INTRODUCTION

In a globalized world, buying meat should not be a gambling experience for the consumer because competiveness in price and quality of the product being sold is vital to the meat retailer remaining in business. Therefore, precise description and quality assurance of the meat product should be a priority for industry and governments in order to deliver well defined quality groups to the appropriate markets in the world.

In this first chapter of the thesis, a perspective of the Canadian beef industry will be offered through review of the Canadian cattle inventories, farm cash receipts and the importance of the beef meat industry in Canada. Also, red meat consumption trends will be reviewed to provide a comprehensive vision of how international markets are and will remain important to maintain the prosperity of the Canadian beef industry. The economics that govern the meat market also need to be understood so that the importance of quality is appreciated (Sections 1.2, 1.2.1 and 1.2.2). Changing and controlling beef quality requires understanding of skeletal muscle structure, composition, and factors and/or practices that affect its quality, as well as traditional and new methods to measure and/or predict

beef meat quality. Moreover, a review of how countries with formal beef meat grading systems are discussed in comparison to the Canadian beef grading system, with consideration of its ability to predict beef meat eating quality.

1.2 IMPORTANCE OF BEEF CATTLE INDUSTRY

The global beef cattle inventory as of January 2012 was approximately 1 billion head of cattle. Leading the world cattle inventories at that time was India with 277.8 million head followed by Brazil with 185 million head, China 103.9 million head, and the United States of America with 91.5 million head. Canada's cattle inventory as of July 1st, 2012, was 13.5 million head for all cattle operations and accounted for approximately 1.2% of the world's inventory (Anonymous, 2012a). The Canadian cattle inventory has decreased 9.32% since 2008, at which time Canada's inventory reached a peak of 14.9 million head in all cattle operations on 96,430 Canadian farms and ranches distributed in Canada. The province of Alberta is the most important contributor to the Canadian cattle inventory, with cattle numbers that accounted for 40% of Canada's cattle population as of July 1st, 2012, at 5.41 million head. The cattle industry generated significant revenue in Canada, totaling $6.2 billion in 2010, which constituted 14% of total farm cash receipts. Of the $6.2 billion in beef industry receipts, $ 3.08 billion were generated in Alberta, accounting for 49% of total cattle farm cash receipts in 2010 (Anonymous, 2012b).

Slaughter capacity in Canada is segregated into federal and provincial abattoirs, with federally inspected abattoirs eligible to export

product, while provincial abattoirs are limited to within province distribution. In 2011, Canada had 22 federally inspected cattle slaughter plants but only 18 were operating, with 5 located in Alberta. Federally-inspected abattoirs account for the largest proportion of the Canadian cattle kill, and in 2011 2,889,627 cattle were slaughtered at federally-inspected establishments in Canada with 1,631,106 or 56% of them slaughtered in Western Canada (Alberta) while 168,023 cattle were slaughtered in provincially-inspected establishments. Of the cattle slaughtered at provincially-inspected abattoirs, 27,993 were slaughtered in the province of Alberta (Anonymous, 2011a). This low number (27,993) of processed cattle is mostly likely due to the tendency of provincially inspected establishments to process cattle based on small beef producers' custom orders, which supply small local niche markets.

1.2.1 Beef Cattle Industry in Alberta

The province of Alberta is an important contributor to the Canadian beef industry because it contains 2.23 million breeding beef cows and heifers, which constitute 39% of Canada's breeding cattle inventory. Traditionally in Alberta, calving occurs during the months of February, March and April, although some producers practice autumn calving (September, October and November). In traditional operations, calves are raised with their dams (mothers) and graze on pasture during the Northern hemisphere spring, summer and fall (April to November). Calves are weaned from their dams from September to November, when calves weigh about 160 kg to 295 kg, depending upon several factors including their age, genetics and quality of their diet. Calves are then

sorted based upon body type into early or late finishing calves, with the early finishing calves characterized as "growthy", muscular calves and late-finishing calves being short and lacking muscling. Calves that are deemed late-finishing are fed with a high forage diet to increase their weight to about 340 kilograms before they are placed on a feedlot finishing program and this process is usually called ''back-grounding''. After back-grounding is completed, cattle are placed on a feedlot finishing program that normally consists of feeding the cattle finishing rations that have high grain concentration for at least 100 days. Steers are normally processed at 525 to 600 kilograms, and heifers at 475 to 525 kilograms. At this stage animals are 12 to 24 months of age (Anonymous, 2012c).

1.2.2 Meat Consumption Trends

The Canadian red meat industry, which includes beef, pork, lamb, meat goats, rabbits, venison and bison, is the largest sector of the food manufacturing industry in Canada with shipments worth $24.2 billion in 2010 (Anonymous, 2011a). In 2009, beef was the most consumed meat in Canada with 12.1 kg per capita whereas chicken was 10.53 kg per capita, 9.66 kg per capita for pork, mutton and lamb was 0.46 kg per capita, veal was 0.43 kg per capita and offal was 0.77 kg per capita. Despite beef being the most consumed meat in the Canadian market, the consumption of beef has decreased steadily since 1981(Anonymous, 2010) and is expected to be 11.84 kg per capita by 2020 resulting in a decline of 14% since 1984 when beef consumption reached a peak of 17.37 kg per capita (Anonymous, 2011a). Beef consumption in Canada is negatively correlated with poultry consumption ($r = -0.78$), the median age of the Canadian population and

its ability to pay for food (r = -0.84), general population growth (r = -0.87), food prices (r = -0.87), immigration into Canada (-0.93) and Canadian population health concerns (r = -0.76) (Agriculture and Agri-Food Canada [AAFC], 2011b). As a result, beef consumption is expected to continue to decrease because the median age of the Canadian population will continue to increase, and the Canadian general population is also anticipated to increase to 35.4 million by 2020 primarily through immigration from Asian countries, which traditionally prefer chicken and pork. Canada Beef Inc., the beef marketing agency for Canada, in its 2013-2014 Marketing Plan Summary, indicated that "having Canadian beef recognized for its premium quality, safety and value in priority markets" and "to enhance Canadian beef brand loyalty" were among its objectives (Canada Beef Inc., 2013). To control beef quality and achieve the desired market success, a full understanding of the actual beef quality represented by the Canadian beef grading system as well as what factors control beef quality is needed. To do this, a review must begin with the structure of meat.

1.3 SKELETAL MUSCLE STRUCTURE AND FUNCTIONS

In order to transmit force originating in the myofibrils to the entire muscle and ultimately to the structure that is moved, skeletal muscle has a very complex organization. A thick structure made of connective tissue called epimysium encloses the entire muscle, and in most muscles the epimysium is continuous with the tendons that connect muscle to bone. Muscles are integrated by bundles of muscle cells (also known as fasciculi) which are surrounded by the perimysium and another structure called

endomysium surrounds the muscle cells. This endomysium overlies the muscle cell membrane, which is known as the sarcolemma (Bailey *et al*., 1989).

The skeletal muscle fiber is a multinucleated, membrane-bound cell and its diameter ranges typically from 10 to 100 μm (Bottinelli *et al*., 2000). When muscle fibers are observed through a microscope, transverse striations can be observed. These striations are caused by specialized contractile organelles called myofibrils. These striations are the result of alternating protein dense A-bands and opaque I-bands within the myofibrils. Bisecting the I-bands are dark lines known as Z-lines, which are protein structures to which the thin filament proteins including actin are anchored. The area between two Z lines is known as the sarcomere and the sarcomere is considered the basic contractile unit of muscles. Sarcomeres are present in series of thousands in a myofibril (Pearson *et al*., 1989) and individual sarcomere size ranges from 1.3 to 3.0 μm long (Akers *et al*., 2008). The less dense I-band is composed primarily of thin filaments while the A-band consists of thick filaments and some overlapping thin filaments (Goll *et al*., 1984). The backbone of the thin filaments is formed primarily of actin protein molecules while the largest component of the thick filament is the myosin protein. The protein myosin contains an alpha helical tail or rod region that forms the backbone of the thick filament and a globular head region that extends from the thick filament and interacts with the actin in the thin filament. The *rigor* complex formed by the interaction of myosin and actin is often referred to as actomyosin. When contracted muscle or *post rigor* muscle is observed in electron micrograph images, the actomyosin have the appearance of cross-bridges between the

thick and thin filaments and, indeed, it is often referred to as such. In *post mortem* muscle these bonds become irreversible and are also known as *rigor* bonds. The globular head of myosin has enzymatic activity; it can hydrolyze the high energy molecule adenosine triphosphate (ATP) and liberate useful energy. The ATPase activity of myosin in living muscle during contraction provides energy for myosin bound to actin to swivel and sequentially pull the thin filaments toward the center of the sarcomere. This action contracts the myofibril, the muscle cell and eventually the whole muscle. The myosin and actin can separate when a new molecule of ATP is bound to the myosin head (Goll *et al*., 1984).

1.4 BEEF MEAT QUALITY

Defining meat quality is challenging due to the multiple complex factors that influence it. At the retail level, intrinsic meat quality characteristics include meat colour, shape, appearance, tenderness, juiciness, flavor, aroma, nutritional value, and safety, while extrinsic characteristics of price, animal welfare, traditions, carbon footprint, brand name, origin, packing, labeling, and traceability can affect consumer product approval (Hocqette *et al*., 2012). Properties of meat such as pH, fat binding capacity, protein solubility, water holding capacity (WHC) and shear force are essential for value-added meat processors to maintain quality and profitable yields of their products (Allen *et al*., 1998; Lopez-Bote *et al*., 1989).

1.4.1 Tenderness

Meat tenderness may be defined either as ''an inherent property of meat determined by its composition and structure,'' or as ''the ease with which a piece of meat disintegrates on chewing'' (Szczesniak and Torgenson, 1965). Research has indicated that meat tenderness is the most important quality trait for the consumer (Verbeke *et al*., 2010; Savell *et al*., 1987) and has shown that consumers are willing to pay an increased price for guaranteed tender meat (Platter *et al*., 2005; Miller *et al*., 2001). This has led to research that focuses on understanding and measuring beef tenderness. Tenderness is extremely variable among carcasses, between muscles and will vary even within the same muscle (Alsmeyer *et al*., 1965; Sharrah *et al*., 1965; Smith *et al*., 1969; Wheeler *et al*., 2007). Tenderness is also affected by cooking methods and endpoint temperature as reported by Obuz *et al* (2004). In a study by Wulf *et al* (1998) shear force values were related to consumer preference and divided into three categories: 1)"Tender" for shear force values of < 3.5 kg; 2) "Acceptable" for shear force values ranging from 3.6 – 4.9 kg; and 3)"Tough" for shear force values >5.0kg . In a recent study in Venezuela, meat that had Warner-Bratzler shear force values of less than 37.98 Newtons (3.87 kg) was considered tender, while "tough" meat had Warner-Bratzler shear force values greater than 37.98 N (Rodas-González *et al*., 2009).

According to Koohmaraie *et al* (1987), meat tenderization begins immediately after exsanguination, with proteolytic degradation of myofibrils by calpain-1, a Ca^{2+}-dependent protease (CDP-I) (Smulders *et al*. 1992). Cooked beef muscles are toughest 9 to 24 hours *post mortem*, before calpains have had sufficient time to affect muscle protein linkages

(Koohmaraie, 1996). The cause of toughening during the first 24 h has not been definitively determined, although Goll *et al* (1995) reported that the change in strength of the binding state of the actin/myosin interaction may cause severe shortening in the first 24 hours *post mortem*. This interaction may be indicated by the myofibril fragmentation index, a method which has been shown to be useful in characterizing tenderness groups (Culler *et al*., 1978; Parrish *et al*., 1979). Culler *et al*. (1978) claimed that the myofibril fragmentation index accounts for more than 50% of the variation in strip loin steaks, and that the myofibril fragmentation index indicated tenderness more strongly than collagen solubility or sarcomere length. Moreover, several authors reported that sarcomere length does not affect tenderness (Culler *et al*., 1978; Seideman *et al*., 1987; Shackelford *et al*., 1994; Smith *et al*., 1979); however, the majority of studies suggested that sarcomere shortening is responsible for the decrease in tenderness of muscles from the time of slaughter to 24 h *post mortem*. Bouton *et al* (1973) observed significantly shorter sarcomere lengths in the *m. semimembranosus*, *m. gluteus medius*, *m. biceps femoris*, and *m. longissimus dorsi* muscles, as well as in the tough *m. adductor* and *m. vastus lateralis* muscles conditioned *post mortem* at 0 to 1 °C than in those same muscles conditioned at 15–16 ° C. The authors also observed strong relationships between Warner–Bratzler shear force values and sarcomere lengths when sarcomeres were shorter than 2.0 μm. In addition, Bouton *et al*. (1973) reported that shear force values decreased exponentially as sarcomere lengths increased. Davis *et al* (1979) reported that when comparing ''less tender'' and ''more tender'' steaks, ''more tender'' steaks had longer sarcomeres (based on mean values) than ''less tender'' steaks. Davey *et al*. (1967) observed that myofibrillar contraction state specifically contracted

to 40% of resting length affected ageing potential, resulting in no effect of aging on tenderness.

Along with sarcomere length, enzymatic degradation can be affected by several factors including: temperature, pH, muscle fiber type, amount and degree of cross-linking in connective tissue, and animal species. Proteolytic enzymes such as calpains and lysosomal proteases are considered responsible for enzymatic degradation (Smulders *et al*., 1992). The aging process usually required 1 to 2 days in chicken, 3 to 6 days in pork, and 10 to 20 days in beef (Smulders *et al*., 1992).

1.5 FACTORS THAT AFFECT BEEF MEAT QUALITY

The factors that affect meat quality can be divided into two categories: the fixed factors linked to the animal that include breed, age, and gender, and the random factors linked to the animal such as diet, weather, and slaughtering procedures (Priolo *et al*., 2001). The quality of meat in most cases can be described by its pH, water holding capacity (WHC), colour, shear force or by performing proximate analyses on the meat sample.

1.5.1 Composition and Location of Water in the Muscle

Lean muscle is composed of approximately 75% water, 20% protein, 5% lipids, 1% carbohydrates and 1% vitamins and minerals, with the latter often characterized during proximate analysis as ash (Offer and Cousins, 1992). In muscle the majority of the water is held within the structure of the muscle and muscle cells, with water found within and

between the myofibrils and the cell membrane (sarcolemma), between muscle cells and between muscle bundles (groups of muscle cells) (Offer and Cousins, 1992.; Honikel, 2004).

1.5.1.1 Bound Water

Water is a dipolar molecule and because of its nature water molecules are attracted to charged species such as proteins. Water in small fractions, approximately 0.5 g of water per gram of tissue, can be found tightly bound to proteins in muscle cells (Offer and Knight, 1988). By definition, bound water is the water that is bound covalently to non-aqueous constituents like proteins and this water has no mobility. Bound water is resistant to freezing and to being evaporated by conventional heating (Fennema, 1985). Because the total concentration of protein in muscle is approximately 200 mg of protein/g of muscle, this bound water makes up less than a tenth of the total water in muscle. The amount of bound water rarely changes if at all in *post rigor* muscle (Offer and Knight, 1988).

1.5.1.2 Immobilized Water

Another fraction of water that can be found in muscle is termed entrapped or immobilized water (Fennema, 1985). The water molecules in this fraction are not bound to protein and may be held either by steric effects and/or by attraction to the bound water (Offer and Knight, 1988). Although this water does not flow freely from the muscle early *post mortem*, it can be removed by drying, and can be also converted to ice during freezing. This water can eventually escape as purge mainly when

alteration of muscle cell structure and pH lowering occurs as the *rigor* process and the conversion of muscle to meat take place (Offer and Knight, 1988). Immobilized water is located between the salt-soluble proteins (actin, myosin) of muscle intermolecular space where it is held in place by capillary force. The space between these proteins varies between 320 Å and 570 Å depending on sarcomere length, pH, ionic strength and osmotic pressure. Changes in pH alter myofilament protein net charge, which will alter the spatial arrangement of the myofilaments and as a consequence alter the amount of water immobilized in this compartment (Brewer, 2004). When hydrogen ions accumulate in the muscle during the conversion of muscle to meat, the pH declines to near the isoelectric point of the meat proteins (especially myosin), which is about pH 5.0 to 5.1 (Béchet *et al.*, 1989). At this point, proteins are attracted to each other, resulting in a reduction in the amount of water that can be attracted and held by that protein. Additionally, because like charges repel, as the net charge of the proteins that make up the myofibril approaches zero (diminished net negative or positive charge) repulsion of structures within the myofibril is reduced allowing those structures to pack more closely together. The end result is a reduction of space within the myofibril, which means less water-holding capacity in meat (Offer, 1991).

1.5.1.3 Free Water

Free water is water that flows freely in the muscle. The flow of this water is mainly affected or held by weak immobilized water surface forces in meat. Free water is not easily seen in pre-*rigor* meat, but can develop as conditions change that allow the immobilized water to move from the

structures where it is found (Fennema, 1985). After 24 to 48 hours *post mortem*, drip channels are formed by gaps between the fibre bundles and the perimysial network, which then facilitate the release of water from the muscle (Offer and Cousins, 1992).

1.5.2 Connective Tissue Content in Muscles

It has been proposed by several authors that connective tissue is one of the main factors that contribute to beef toughness (Bouton *et al*., 1975; Koohmaraie *et al*., 2002; Møller, 1980). Bendall (1967) reported that collagen content varied greatly between muscles by quantifying the amount of collagen of several muscles based on dry weight. This author found that low collagen content muscles included the m. *gluteus medius* at 1.6% collagen dry weight, the m. *adductor* at 1.7%, the m. *pectineus* at 1.9%, and the m. *quadriceps rectus femoris* at 1.9%, whereas the high collagen content muscles included the m. *sternomandibularis* at 7.3%, m. *panniculus* at 9.7%, and the m. *peronaeus tertius* at 15.1%. Torrescano *et al*. (2003) reported a correlation between the amounts of total and soluble collagen with the tenderness of raw muscles. According to Prost *et al*. (1975), functions of muscle seems to affect collagen content, and the author observed that muscles of the forequarter were found to have higher connective tissue content than those of the hindquarter. This was further explained by the forelimb muscles being connected to the body through muscular attachments, unlike the hind limb muscles which are attached by bones. Thus, muscle collagen contents can greatly differ and these differences should be taken into account when comparing results between muscles. Bendall (1967) also observed that collagen content varied within

a muscle due to the biochemical transmission of forces necessary for locomotion. The m. *trapezius thoracis* is formed by a thick section, which contain 4.0% and a thin section containing 8.6% of collagen of the dry muscle weight. Moreover, the anterior end of the m. *pectoralis superficialis* contained 4.0% of collagen and the posterior section contained 7.2%. A study by Shackelford *et al*. (1997) agreed with the findings of Bendall by showing that Warner-Bratzler shear force increased significantly from 3.87 to 5.07 kg starting from the distal end and ending at the proximal end of the m. *semitendinosus* with a mean shear force of 4.05 kg.

Finally, collagen muscle content is, to a certain extent, inversely proportional to age. Dikeman *et al*. (1986) acknowledged in their study that young cattle generally have more collagen per gram of wet muscle tissue than mature cattle and also observed that the quantity of collagen appeared to stabilize with age. Hence, Gerrard *et al*, (1987) reported that collagen content declined during growth outwardly because it is diluted by the increase in the size of the muscles through the addition of proteins and fat.

1.5.3 Rigor Mortis and Post Mortem Glycolysis

Rigor mortis is an unmistakable sign of muscle death. This phenomenon is caused by the biochemical changes in the muscle after the animal dies, causing stiffness in muscle. There are three phases of *rigor mortis* in the animal carcass: delay phase, onset and resolution (Bendall, 1973b: 244). During the first phase of *rigor mortis*, known as the delay phase, adenosine triphosphate (ATP) concentrations in muscle are sustained by the metabolism of creatine phosphate (CP), which donates its

phosphate to adenosine diphosphate (ADP). ATP is needed to relax muscles by disengaging the binding site between actin and myosin. Early *post mortem* there is sufficient ATP to allow the muscle to react and appear as if it was alive (soft, extensible and reactive to electrical stimulation) (Bendall, 1973b: 246). When the concentration of ATP reaches about 1/2 of the original value of resting muscle, *rigor* is triggered (Bendall, 1973b: 289). In rested well fed animals, the CP is present in sufficient concentrations to maintain high levels of ATP; however, many animals that have experienced inanition and/or exhaustion have little or no CP in muscles at the time of slaughter. Therefore, ATP will be then generated from glycogen thru anaerobic glycolysis, thus keeping meat in the pre-*rigor* state (Bendall, 1973b: 267-268).

The appearance, functionality and quality of meat can be determined by the rate of pH decline *post mortem* and its interaction with temperature. Animals with low levels of muscle glycogen at the time of slaughter will have a shortened delay phase and go into *rigor* faster than normal (alkaline *rigor*), and this problem is frequently found in animals that have been under stress before slaughter such as cattle mixed with unfamiliar cattle or hunted animals. When there is not enough glycogen, hydrogen ions will not be produced in sufficient amounts to produce a decline in muscle pH, and the high pH will promote the appearance of dark, firm and dry beef (Bendall, 1973b:252). During the onset of *rigor mortis*, stiffening and loss of extensibility of muscles will occur as a consequence of the failure of the calcium pump to return calcium to the sarcoplasmic reticulum. This failure will result in the accumulation of Ca^{2+}

ions inside the cell allowing actin and myosin to bind and contract (Bendall, 1973a).

Fat cells break down triacylglycerols into fatty acids in a very slow process, therefore, free fatty acids cannot be utilized for the creation of other energy-rich compounds such as ATP because the two-carbon units (–CH2–CH2–) required for acetyl-CoA (CH3– CO–S–CoA) need oxygen, which is not available *post mortem*. This explains why fats remain relatively unchanged in the meat. At this stage muscle cells can still use glycogen as their energy source but due to the lack of oxygen the citric acid cycle and the oxidative phosphorylation pathways no longer function. As a result, pyruvate is no longer decarboxylated and is reduced to lactate. This is considered the termination of the anaerobic breakdown of glycogen in the muscle *post mortem* (Honikel, 2004).

After exsanguination, glycolysis continues without oxygen and as a result of this anaerobic glycolysis lactic acid and hydrogen ions are produced. The extent of accumulation of hydrogen ions and the resultant pH decline in meat are dependent mainly on the concentration of glycogen present in the muscle at time of slaughter (Price and Schweigert, 1987); therefore, glycogen concentration can be used as a means to determine or predict the ultimate pH of the meat. Glycolytic potential, which is a measure of lactate combined with potential lactate produced from glycogen, reflects the usual amount of the steady state of glycogen stored in muscle that could be converted into lactate, plus the lactate concentration at time of sampling. Therefore glycolytic potential is less sensitive to sampling time and sample handling than either glycogen or lactate measured alone (Hartschuh *et al*., 2002), consequently glycolytic

potential it has been used by several researchers to study the relationship between muscle glycolytic potential and meat quality of cattle (Immonen *et al*., 2000), and pigs (Hambrecht *et al*., 2004; Hambrecht *et al*., 2005), respectively.

1.5.4 Early *post mortem* carcass management

Muscle to meat conversion is completed when muscles have depleted energy reserves or have lost the ability to use the remaining reserve. The length of time before *rigor mortis* is completed is important in the determination of the meat quality. This time varies between species; *rigor mortis* is completed at 4 hours in chicken carcasses, 10 hours for pig carcasses, 15 hours for lamb carcasses, 18 hours for calf carcasses, and 29 hours for beef carcasses at a temperature of 15°C (Etherington *et al*., 1987).

Early *post mortem* temperature has an effect on glycolytic enzyme activities which will affect the rate of glycolysis. High early *post mortem* temperatures have a positive effect on muscle glycolytic enzyme activities, accelerating pH decline in muscles. Conversely, low *post mortem* temperatures have a negative effect on glycolytic enzyme activity, slowing the glycolysis rate and muscle pH decline (Pearson and Young, 1989). Bendall (1973b) observed that *rigor mortis* of muscles from carcasses of exhausted animals that were maintained at 37°C can last from less than half hour to four hours with well-fed animals, whereas one to ten hours may be required for those types of animals when the carcasses are stored at 20°C. Bendall (1978) reported that glycolytic rate differences also existed between muscles types. A later study by Tarrant (1989) confirmed the results observed in the Bendall study and reported that glycogenolysis can

also vary between muscles and fibre types. These phenomena are caused by pre-slaughter physical activity (cattle handling) and adrenal activation (Clark *et al*., 1997). In cattle, muscles located in the back and the hind limbs are the most affected by glycogen depletion (Tarrant and Sherington, 1980).

Pre-slaughter stresses have been found to affect muscle concentrations of high-energy inorganic phosphate (P_i), ATP, ADP, and phosphocreatine. As observable in beef, the exhaustion and or stress of cattle before slaughter can cause the reserves of glycogen in muscle to be depleted and remain at a low level at the time of slaughter. This lack of glycogen will cause dark, firm, and dry (DFD) meat (Pearson and Young, 1989).

Early *post mortem* carcass temperature will influence the speed of the chemical reactions that occur in meat, especially during the time muscle is under the delay phase of *rigor mortis* as example: At a *post mortem* storage temperature of 17°C, muscle of a well fed, immobilized rabbit required almost 11 hours to enter *rigor*, yet when a portion of the same muscle was held at 38°C *post mortem* it required only 5 hours to enter in *rigor* (Bendall, 1973b). At the resolution of *rigor mortis*, muscle will relax not because ATP has relaxed the bond between actin and myosin as happens *in vivo* but because of degradation of proteins by endogenous proteolytic enzymes (Bendall, 1973b).

1.5.5 Cold Shortening

Cold shortening is a condition in the carcass muscles where the sarcomere is shortened early *post mortem* by exposure to chill temperatures

such that the temperature of the muscle is reduced to below 10°C while ATP is still present (Hannula and Puolanne, 2004). Cold induced shortening or cold toughening represents a serious economic concern for the beef industry, due to the negative impact on meat tenderness (Hamby *et al*., 1987). Conditions required to minimize cold shortening include maintaining constant chill temperature conditions (avoid temperature fluctuations), minimal air circulation, and a high relative humidity (Smith and Carpenter, 1973). The earliest research on cold shortening dates from the 1960s when Locker *et al*. (1963) defined cold shortening as the rapid decline in muscle temperature to less than 14 to 19 °C before the onset phase of *rigor mortis*. Subsequent research showed that when muscle temperature was reduced to between 0 and 15 °C before the onset phase of *rigor mortis*, the sarcoplasmic reticulum was unable to retain calcium due a decrease in ATPase activity which led to an abundance of calcium in the sarcoplasm (Hannula *et al*., 2004). The presence of ATP in the muscle enabled the contraction of the muscle at a maximum level, causing the filaments to slide over one another obliterating the I-band of the sarcomere. The sarcoplasmic reticulum appears to be least functional when muscle temperatures are between 1 and 2 °C in the early *post mortem* period (Aberle *et al*., 2001).

The relationship between temperature and pH at the beginning of *rigor mortis* is a decisive factor in determining cold shortening potential (Hannula *et al*., 2004). Cold shortening susceptibility varies between muscle types, with red muscles being more susceptible than white muscles (Bendall, 1973a) due the high amounts of glycogen that white muscle fibers sequester. As a result, this muscle type experiences a sharp

decrease in pH earlier than red muscle fibers in the *rigor mortis* process. Pork is comprised primarily of white muscle fibers, which makes it less susceptible to cold shortening than beef and lamb, which have large proportions of red fibers. This demonstrates that muscles are not affected to the same degree by cold shortening because the interactions among time, temperature, and pH differ between muscle types and between species, and can differ within large muscles (Hannula *et al*., 2004).

Sarcomere shortening is also characteristic of the meat quality defects known as thaw and heat *rigor*. These phenomena are caused when carcasses are exposed to extreme cold or hot temperatures pre-*rigor*, respectively. Thaw *rigor* develops when muscle that was frozen pre-*rigor* mortis is thawed. When muscle is thawed, contraction is produced by the abrupt release of Ca^{2+} into the sarcoplasm which produces a physical shortening of 60–80% of the original muscle length, prompting the release of large quantities of meat purge and severe muscle toughening. Heat *rigor* occurs when muscles are maintained at elevated temperatures up to 50 °C resulting in an accelerated depletion of ATP, which will create severe shortening and the early onset phase of *rigor mortis* (Aberle *et al*., 2001).

1.5.6 Carcass Fatness

Carcass weight and composition are considered important factors affecting chilling rate (Kastner, 1981). Carcass fat thickness can reduce the incidence of cold shortening in beef (Dolezal *et al*., 1982) and lamb carcasses (Smith *et al*., 1976). Smith *et al*. (1976) observed that increased thickness of subcutaneous fat improved tenderness by allowing the

carcass to chill slowly, which facilitated enzyme activity. Increased carcass fatness appears to decrease chilling rate by either increasing the amount of carcass insulation or by increasing carcass total mass. Dolezal *et al*. (1982) reported that carcasses with 2.54 mm of external fat received the lowest sensory panel ratings for myofibrillar tenderness and had the highest shear force values but as fat thickness increased up to 7.61 mm, palatability also increased. Smith *et al*. (1973) acknowledged that a fat covering of 2.5 mm in lamb carcasses prevented excessive *post mortem* shrinkage during chilling and transit. Tatum *et al*. (1982) observed that lean carcasses had decreased tenderness and also identified a threshold of 0.76 cm of back fat as critical for compromised meat quality.

1.5.7 Carcass Electrical Stimulation

The process of applying an electrical current through a carcass before the onset stage of *rigor mortis* occurs is called electrical stimulation (ES). When applied, electrical stimulation reduces the susceptibility of pre-*rigor* muscle to cold shortening by inducing vigorous muscular contractions throughout the carcass. These contractions promote the use of energy (ATP) by the muscles, and in response to the increased energy expenditure a dramatic acceleration of pH decline will follow. Electrical stimulation of a beef carcass usually reduces the muscle pH by 0.5 units within 1 minute of electrical stimulation. A similar decline of pH (0.5 units) in a carcass that does not receive electrical stimulation usually requires three or more hours (Ducastaing *et al*., 1985).

Electrical stimulation of beef carcasses has significant positive effects on lean maturity score, overall maturity score, and Warner–Bratzler

shear force values (Calkins *et al*., 1980; Savell *et al*., 1978). There are two accepted mechanism of action of electrical stimulation on meat tenderization: the first is by myofibrillar disruption and tearing due to the extreme muscle contractions caused by electrical stimulation (Marsh, 1986); and the second is the tenderization effect of electrical stimulation in beef due to the accelerated pH decline (Chrystall and Devine, 1985; Chrystall and Hagyard, 1975; George *et al*., 1980). Electrical stimulation accelerates the muscle pH decline in the early *post mortem* period while the carcass temperature is high so that the speed of the tenderization process is faster than in a carcass in which *rigor mortis* has been delayed until the carcass temperature is low. The electrically stimulated carcass therefore will have a time advantage in achieving its ultimate tenderness if it has a combination of lowered pH and high temperature early *post mortem*. The electrically stimulated and non-stimulated carcasses will achieve the same ultimate tenderness, however, with time; simply stated, stimulated carcasses achieve ultimate tenderness faster than non-stimulated carcasses (Chrystall and Daly, 1996). If lengthy (greater than 60 seconds) high voltage (greater than 400 volts) electrical stimulation is applied to beef carcasses, an excessively rapid pH decline will occur while the carcass has a high (greater than 20 °C) temperature causing adverse effects on tenderness, colour stability water holding capacity and other meat quality traits (Geesink, *et al*., 2001; Koh, *et al*., 1987; Ledward, *et al*., 1986; Unruh *et al*., 1986).

Another explanation for how electrical stimulation increases tenderness involves low heat shock proteins (Hsp), which are a family of proteins that protect other proteins against degradation and structural

damage from the natural occurring apoptotic processes in cells (Beere, 2005). Two heat shock proteins (Hsp27 and Hsp70) have been identified that directly inhibit both the intrinsic and extrinsic apoptotic pathways (Gotoh *et al*., 2004; Paul *et al*., 2002). Bjarnadottir *et al*. (2011) reported that electrical stimulated muscles had a lower abundance of Hsp70 than non-electrical stimulated muscles, and implied that electrical stimulation may accelerate apoptosis and *post mortem* tenderisation indirectly by reducing the effects of Hsp70.

Colour changes in meat due to the action of electrical stimulation are the result of the interaction between temperatures and pH on the oxygen consumption rate by proteins of the electron transport chain located in the inner mitochondrial membrane. The combination of muscle pH and temperature affect the extent of denaturation of these proteins and therefore their reductive capacity. Non-mitochondrial enzymes appear to be involved in the reductive reactions in the muscle as well (Bernas and Dobnicki, 2002; Takahashi *et al*, 2002.) Reduction of the tetrazolium salt 3-(4, 5-dimethylthiazol-2-yl) 2, 5-dyphenyltetrazolium bromide (MTT) can be used to measure the reductive capacity of meat because this salt is an indicator of reduction-oxidation activity in cells. Using the tetrazolium salt MTT with the right reduction potential, one could assess the activity at each specific stage in the mitochondrial electron transport chain. This reduction of meat as assessed by MTT reduction is linked to oxygen consumption rate and cytochrome b5 dependent metmyoglobin reductase enzyme activity (Berridge *et al*., 1993).

1.5.8 Carcass Suspension Methods

Different carcass suspension methods have been used to prevent sarcomere shortening and tenderize specific cuts of beef. A study done by Hostetler *et al.* (1972) compared results of several suspension methods such as vertical hanging (standard Achilles-tendon suspension), horizontal (side placed on table, with limbs tied perpendicular to the vertebra), neck-tide (side suspended from the cervical vertebra, with pelvic limb tied to thoracic limb), hip-free (side suspended from the obturator foramen and pelvic limbs free), and hip-tied (side suspended from the obturator foramen with the thoracic limb tide to the pelvic limb). The hip-free method was reported to have the most positive impact in increasing tenderness as a result of lengthened sarcomeres, especially in the loin and round muscles. Hostetler *et al.* (1975) reported that suspending carcasses by the obturator foramen (i.e., Tenderstretch) resulted in muscles with 17% longer sarcomeres lengths than those from carcasses suspended using the standard Achilles-tendon suspension, and that this reduced the overlap of thick and thin filaments. In a recent study, Janz *et al.* (2006) reported that altered carcass suspension (*obturator foramen*) reduced shear force of the *longissimus lumborum* muscle by 25% accompanied by 28% increase in sarcomere length.

1.5.9 Carcass Chilling Methods

1.5.9.1 Delayed Carcass Chilling

Delayed chilling is a technique that involves keeping intact carcasses out of the chill room for some period of time early *post mortem*.

Delayed chilling should not be confused with high temperature conditioning of muscles, which is perform by subjecting primal cuts to elevated temperatures after boning (Smulders *et al*., 1992). Marsh *et al.* (1980–1981) reported that beef sides processed by delayed chilled at 37 °C for 3 h *post mortem* were found to be more tender than conventionally processed chilled sides. Smulders *et al*. (1992) reported that prevention of cold shortening and increased proteolysis were the potential benefits of high temperature.

If delayed chilling is not performed correctly, it can result in ''heat shortening'' which toughens muscles. Smulders *et al*. (1992) stated that the effect of increased tenderness of the delay-chilled muscle can disappear during storage, indicating that delayed chilling serves to accelerate ageing. The practice of delayed chilling may also induce bacteria proliferation on carcass surfaces due the warm conditions that this process requires. Therefore, delayed chilling is not practiced commercially in the U.S. or Canada because bacterial proliferation on carcass surfaces under such conditions is a serious health concern for human consumption (Smith *et al*., 2012).

1.5.9.2 Carcass Spray Chilling

Livestock carcasses are chilled so that food safety is ensured, maximum shelf life maintained and shrinkage of muscles reduced, with little focus on maintaining tenderness and colour of the finished product (Savell *et al*., 2005). Rapid chilling processes include ''spray chilling'', which consists of intermittent water sprays (Brown *et al*., 1993), mainly by replacing moisture lost by evaporation (onto carcasses in the chiller

usually in first 3 to 8 h *post mortem*) (Hippe *et al*., 1991). Spray chilling has been reported to reduce carcass weight loss by approximately 2% (Gigiel *et al*., 1989) especially during the first 24 h *post mortem* (Allen *et al*., 1987). When spray chilling is applied, the surface remains wet allowing maximum mass transfer and evaporative cooling without increasing carcass weight loss (Gigiel *et al*., 1989). The spray chilling system is currently used throughout the world for beef, lamb, poultry (Brown *et al*., 1993), and also pork (Gigiel *et al*., 1989).

During the first 24 h of conventional chilling of beef, pork, and lamb carcasses, evaporative weight losses can be as much as 2% of the hot carcass weight (Greer *et al*., 1997). Smith *et al.* (1973) conducted a study on 1000 lamb carcasses, and reported that of the total weight loss due to evaporation and drip that occurs in the first 72 hours *post mortem*, 92 % of occurred during the first 36 hours. The authors also determined that shrinkage continued to increase with time in the cooler, but at a decreased rate and that the majority of the initial carcass weight loss that was experienced in the study was due to evaporative losses of water added during washing of the carcass. Moreover, approximately 39% of the weight loss remaining occurred during the following 60 h due to evaporative losses of moisture from carcass components (Smith *et al.*, 1973). Lamb can be considered susceptible to cold shortening because of the exposed carcass musculature and high surface-to-volume ratio that leads to excessive evaporative weight loss (Brown *et al*., 1993). In commercial practice, there is considerable variation in the duration of spray chilling, and the amount of water deposited on carcasses in a specified period of time is usually not known (Greer *et al.*, 1997). It

appears that spraying carcasses in the initial 3 to 12 h of carcass chilling is relatively common within the commercial setting (Greer *et al.,* 1997). Moreover, spray chilling has also been demonstrated to have a negative impact on important quality attributes such as colour because yellowing of lean muscle colour and sternum fat compared to that of conventionally chilled carcasses has been observed (Brown *et al*., 1993; Greer *et al.,* 1997).

The profit turnover in slaughtering plants is typically between 0.5 and 1% (Collett and Gigiel, 1986); therefore, even a 1% increase in yield is very important for the income of these businesses. For that reason, an important assessment of any chilling system claiming to save weight during the early *post mortem* period is whether the weight loss is simply delayed until later in the storage, distribution and retail chain (Brown *et al*., 1992). Knowing this, the fact that Brown *et al.* (1992) reported no differences between the mean drip losses from the meat at 5 days indicated the ability of the spray-chilled samples to retain their relatively high water content, and this was comparable with the results of conventionally chilled carcass samples.

1.5.9.3 Blast Carcass Chilling

Carcass chilling systems have been extensively investigated so as to rapidly reduce carcass temperatures and decrease carcass processing costs while avoiding cold or heat shortening. In the late 1990's, extremely rapid chilling systems referred to as ''rapid,'' ''ultra-rapid,'' ''blast,'' ''very fast,'' and ''extreme" chilling systems were examined as they were characterized as being able to decrease carcass temperatures to below 5 °C with little or no effect on meat toughness (Savell *et al*., 2005). Some rapid chilling

systems are used in livestock carcasses to comply with government regulations (Bowater, 2001). Brown *et al.* (1992) described ultra-rapid chilling as consisting of two steps; the first step is the air-blast tunnel that works as a pre-chiller, reducing carcass surface temperature to decrease the amount of evaporative weight loss, and the second step is absorbing the initial heat load of the chiller induced by the hot carcasses. Joseph (1996) reported that rapid chilling increases toughening of beef carcasses. Nevertheless, Bowling *et al*. (1987) showed that it is possible to chill beef carcasses at extremely rapid rates with less carcass shrinkage and with no negative effects on quality grade or cooked beef palatability. Aalhus *et al.* (2002) defined very fast chilling as the achievement of a carcass temperature of -1 °C within 5 h of *post mortem* chilling and also acknowledged the ability of pre-*rigor* freezing to produce toughening. The use of blast chilling can result in frozen carcasses that require long periods of time to thaw before fabrication can begin (Watt *et al.,* 1974). In order to prevent cold shortening from happening and produce tender beef some studies paired the use of electrical stimulation with blast chilling. Aalhus *et al.* (2001) acknowledged that by combining blast chilling and electrical stimulation, shear force values in the *m. longissimus lumborum* of beef sides were decreased by 9.5% in comparison with conventionally chilled carcass sides. Aalhus *et al.* (2001) also showed that blast chilling had no effect on marbling score, and also reported that blast-chilled/electrically stimulated carcasses had brighter lean colour than conventionally chilled carcasses. Moreover, lean carcasses in this study had an approximately 0.3% decrease in cooler shrink when blast chilling treatment was applied, and blast chilling was able to reduce the increased drip losses normally associated with electrical stimulation. The result of these studies were

used to recommend to industry the combination of blast chilling and electrical stimulation to reduce *post mortem* chilling times and shrink losses while preserving meat quality (Aalhus *et al*., 2002). Aalhus *et al*. (2002) reported that rapid chilling regimes could produce significantly reduced percentages of cooler shrinkage, an increased perception of marbling, darkened meat colour, and increased drip loss at retail level than conventional chilling. The same authors reported that for -20 and -35 °C blast chilling temperatures, shrink losses in beef decreased as time in the blast chill tunnel increased. After 7 h of -35 °C of blast chilling, cooler shrinkage was totally eliminated, and carcass sides in fact began to gain weight after 10 h of this treatment. Moreover, the combination of 10 h blast chilling with 6 days aging was found to produce the lowest shear values. Aalhus *et al*. (2002) reported that the advantage of using very fast chilling would be a reduction in the necessary aging time to achieve an acceptable product.

1.5.10 Meat Tenderization by Endogenous Enzymes (Ageing)

Due to its biological composition, fresh meat is a highly perishable product. Many interrelated factors influence the shelf life and freshness of meat such as holding temperature, atmospheric oxygen (O_2), endogenous enzymes, moisture, light and most importantly, micro-organisms (Zhou *et al*., 2010).

The most significant effect of *post mortem* ageing is the improvement of meat tenderness, most of which occurs during the first six days *post mortem* (Calkins and Seideman, 1988; Monsón *et al*., 2004). Koohmaraie (1995) observed that there is a large amount of variation in

shear force after only one day of *post mortem* ageing. However, these changes in muscle toughness can continue for 14 (Monsón *et al*., 2005), 21 or as long as 28 days *post mortem* (Gruber *et al*. 2006). The meat tenderization process that occurs during ageing is promoted by calcium-dependent endogenous proteases (calpain), and potentially by lysosomal enzymes (Goll *et al*., 1983; Koohmaraie *et al*., 1988). The calpain system is the underlying mechanism responsible for proteolysis of key myofibrillar proteins during the storage of meat at refrigerated temperatures resulting in meat tenderization (Dransfield, 1999; Koohmaraie, 1996). In the presence of Ca^{2+}, calpain was reported to remove the Z-disc from the skeletal muscle myofibrils and cause several changes in myofibrillar proteins which could be associated with increased meat tenderness (Goll *et al*., 1974). Etherington, (1984) found that the optimal pH range for the activity of calpains is 6.5 to 8.0. Beef steaks from m. *longissimus* with ultimate pH values between 6.1 and 6.5 at one day *post mortem* were significant more tender than those of ultimate pH values of 5.7, a difference explained by the proteolytic enzymes being more active at pH values of 6.1 to 6.5 than at 5.7 (Silva *et al*., 1999). The calpains are present in two distinct forms, μ-calpain requiring 50 to 70 μM Ca^{2+} and the m-calpain 1 to 5 mM Ca^{2+} for activation (Dayton *et al*., 1981; Mellgren, 1980). m-Calpain activity can persist for up to three days *post mortem* without being altered; therefore, its activity remains relatively consistent during *rigor* (Ducastaing *et al*., 1985). Activity of μ-calpain, which is the calpain thought to be mainly involved in the tenderization of meat (Koohmaraie, 1996), decreases by about 50% during *rigor mortis* (Ducastaing *et al*., 1985).

The calpain inhibitor protein calpastatin has also been linked to the extent of tenderization that occurs in beef during *post mortem* ageing. The level of calpastatins has been positively correlated with shear force in bull meat at five and twelve days *post mortem* (Steen *et al*., 1997). Koohmaraie *et al*. (1991) reported that the calpastatin: μ-calpain ratio is around 4:1 in beef m. *longissimus*. Geesink and Koohmaraie (1999) observed that proteolysis by calpains was still occurring at a ratio higher than 4:1; therefore, μ-calpain activity depends on the ionic strength-dependent instability of autolyzed μ-calpain, pH, temperature and the presence of Ca^{2+} .

Due to calcium concentration restrictions and time limitation of their activity, meat tenderization cannot be accomplished by the calpains and the cathepsins alone (Lamare *et al*., 2002). Therefore proteasomes, specifically the 20S proteasome located on the I-band, may also degrade muscle structural proteins, preferentially those located in the Z-discs and I-36 bands (Dutaud *et al*., 2006), and specifically actin, nebulin, and troponin T (Houbak *et al*., 2008).

Proteolysis not only occurs to myofibrils, as protein degradation also occurs in the extracellular matrix to connective tissue. Collagen fibres have a triple-helical structure which makes them very resistant to enzymatic degradation, but there are still enzymes able to degrade collagen. These enzymes are the matrix metalloproteinases, which are zinc-dependant endopeptidases (Kovanen, 2002) that include collagenases, stromelysins, and gelatinases (Sylvestre *et al*., 2002).

1.6 MEAT QUALITY MEASUREMENTS

1.6.1 Traditional Methods and Technologies

Since meat science evolved into a discipline, numerous technologies have been developed or borrowed from other fields to estimate the characteristics of carcasses and the meat they yield. Important carcass measurements incorporated into the Canadian Beef Grading System include prediction of lean muscle yield so that producers may be paid according to the protein produced rather than carcass fat. Research in meat science has focused particularly on describing the texture of meat so that the sensory acceptability of meat products can be predicted without destroying the product by cooking and serving to sensory taste panels. Important methods of assessing the technological qualities of meat are reviewed in this section.

1.6.1.1 Dissection

Dissection is one of the most effective methods to assess the composition of retail cuts or even a whole carcass. Assessment by dissection is highly repeatable in application. This method requires an understanding of anatomy and the patience to carefully separate each component, as well as the ability to prevent or quantify sample weight loss through evaporation and drip. The ability to record carcass section data accurately is also necessary. The dissection method does not account for intramuscular fat, however, which is often assessed visually or by proximate analyses (Kauffman, 2012).

1.6.1.2 Warner-Bratzler Shear Force

In the 1920's K.F. Warner and colleagues (Warner, 1952) proposed the idea of estimating tenderness by shearing a sample of cooked meat, and later this method was improved by L.J. Bratzler changing the specifications of the blade shape, thickness, cutting edge, shearing speed, etc., (Bratzler, 1932). Today Warner-Bratzler shear force (WBSF) is one of the most commonly used instruments to measure beef tenderness in meat science even though this method was found to have variability in tenderness measurement between institutions (Wheeler *et al.*, 2007). To minimize the error in shear force values a protocol has to be follow. Some of the measures used to reduce this error among institutions are: Cooking equipment, cooking the steaks to a constant time and temperature, meat fibre direction (the grain of the meat), diameter of the sample, number of replicates (at least 6 good cores, AMSA, 1995) and adequate sample trimming (free of visible connective tissue or excessive fat) (Wheeler *et al*, 1997). Today most institutions use the "Research guidelines for cookery sensory evaluation and instrumental tenderness measurements of meat" from the American Meat Science Association (AMSA, 1995) in a way to have comparable results.

Warner-Bratzler shear force method is time consuming, it requires a sample to be destroyed in order to assess tenderness and it has a poor repeatability (R^2 = 0.53 to 0.86) (Wheeler *et al*., 1996, 1997). Therefore meat scientists have intensively pursued research on alternative ways of measuring or predicting tenderness accurately.

1.6.1.3 Meat pH

Meat pH is an important characteristic of meat and protein functionality because it influences meat colour, water holding capacity, flavor, tenderness and shelf-life.

Important biochemical changes take place early *post mortem* in muscles that affect the rate and extent of muscle pH decline. During the *rigor* process, the muscle pH of the live animal was originally between 7.0 and 7.2 (Honikel, 2004), and declines to the final or ultimate pH (pHu) of *post rigor* muscle, which ranges from 5.3 to 5.8. Intramuscular pH usually reaches its ultimate value within 6 to 12 h for pork and 18 to 40 h for beef (Smulders *et al*., 1992). The intramuscular pH declines because hydrogen ions form as a by-product of the formation of lactic acid from glycogen in the anaerobic glycolytic pathway. In beef muscles, an ultimate pH from 5.5 to 5.6 is usually achieved by 18 to 36 hours *post mortem*. As explained in section 1.5.3 of this review, reduced glycogen levels mean that the muscle will fail to produce enough lactic acid and hydrogen ions to lower its pH, and this situation will produce the phenomenon of dark cutting beef (Honikel, 2004; Ferguson *et al.,* 2001). A pHu of greater than 6.5 occurs when lactate production is very low (40 μM lactate/g in a muscle with pHu of 6.2) compared to a regular lactate content (100 μM lactate/g) in a muscle with normal meat pH (Maltin *et al*., 2003).

A normal *post mortem* ultimate muscle pH of 5.4 to 5.7 is advantageous because it will prevent or retard microbial growth (Baird-Parker, 1980) and enhance meat flavor development (Montel *et al.,* 1998). Tenderness can also be affected as Dransfield (1981) reported that meat

with a high pHu may be more tender than normal pH meat, possibly because the reduction in glycolytic substrate availability causes a rapid ATP depletion and an early *rigor mortis*, with an early *rigor mortis* reducing the possibilities of carcass cold shortening (Wanatabe *et al*., 1996). Other studies have reported that dark, firm and dry beef has higher shear force values and is less palatable than normal beef (Wulf *et al*., 2002), but the pH of beef in this category is between pH 5.8 and 6.0, a pH range that has been associated with tough beef (Jeremiah *et al*., 1991). Beef that has very low pHu is likely to have poor eating quality as excessive acidification of the muscle causes inactivation of enzymes involved in *post mortem* tenderization, and increased purge and drip loss (Offer and Knight, 1988) resulting in overall low acceptability.

1.6.1.4 Meat Colour

Meat colour is a critical characteristic of fresh meat at retail level, even if the colour has a low correlation with eating quality because meat customers relate a bright cherry red colour with freshness and a brownish or pale colour could result in rejection of the product (Hood and Riordan, 1973). Colour is usually measured using the Commission International De I' Eclairage (CIE) colour system, which is based on three coordinates, L*, a* and b*, where L* measures lightness (100=white and 0= Black), a* measures redness (positive red, negative green) and b* measures yellowness (positive yellow, negative blue) (Commission International De I' Eclairage, 1976).

Myoglobin is the primary pigment of meat and is a complex molecule composed of a protein moiety (globin) and a haem prosthetic

group (Cornforth and Jayasingh, 2004). Myoglobin is located in the cytoplasm of muscle cells and when deoxygenated is purplish in colour. When myoglobin binds to oxygen it becomes oxymyoglobin, which is the myoglobin state responsible for the bright red colour of freshly cut meat, and can be measured objectively by a* coordinates (Priolo *et al*., 2001). This oxygenation or ''blooming'' occurs within 30 minutes when freshly cut meat is exposed to air. In oxymyoglobin, haem iron is in the ferrous state (Fe^{2+}) (Cornforth and Jayasingh, 2004). The remaining colour comes from the haemoglobin which occurs mainly in the circulating blood, but a small amount can be found in the tissues after slaughter (Priolo *et al*., 2001).

Animal age, diet, enzymes and even animal activity can have an effect on meat colour (Priolo *et al*., 2001). No breed effect on colour has been reported (Muir *et al*., 2000). Pasture-fed cattle have been shown to develop yellow fat as a consequence of the high levels of beta-carotene contained by grass. Yellowness of fat can be measured by b* coordinates (Baublits *et al*., 2004). Increased redness of beef cuts could be caused by increased myoglobin, decreased glycogen content in muscle prior to slaughter, or both. When glycogen is depleted rapidly during the transport and handling of cattle just before slaughter, there is minimal hydrogen ion production, which results in dark firm and dry (DFD) meat, and the extent of this condition can be measured by L* coordinates. DFD meat has a low acceptability in the market of table cuts, and shelf life of this meat is reduced due the high pH value which is ideal for bacterial growth (Priolo *et al*., 2001). High pH meat has low L*, a* and b* values, which means meat is darker and less brown than that of normal pH meat (Zhang *et al*., 2005).

Early post mortem temperature and pH can affect beef colour as well, and the temperature and pH decline of muscles on the carcass can be affected by subcutaneous fat cover. Wulf *et al*. (1997) reported that b* values were positively correlated with external fat and L* values had a low correlation to external fat. Page *et al*. (2001) observed that as quality grade increased meat pH declined, colour values increased, and variation in pH and colour decreased. Murray (1989) acknowledged that heifer carcasses produced darker lean than steer carcasses, most likely as a result of an increased cooling rate in the muscles due to differences in muscle mass.

1.6.1.5 Water Holding Capacity of Fresh Meat

Water-holding capacity (WHC) is a very important characteristic of meat because it has a strong influence on product yield, and yield translates into profits because meat is sold by weight. WHC could be defined as the ability of fresh meat to retain its own moisture when force is applied to it such as during the exertion of pressure, cutting, heating or processing. The terms ''drip loss" or "purge loss'' are the names given to the water that drips from raw meat or meat in a package, respectively, whereas water lost during the cooking process of meat is called ''cooking loss''. Meat water losses due to purge can range from 1 to 3% in fresh meat at the retail level (Offer and Knight, 1988). Purge loss can be as high at 10% in pale, soft and exudative (PSE) meat products (Melody *et al*., 2004). Purge loss not only represents a loss of profits due the weight loss, it also represents the loss of a significant amount of water-soluble sarcoplasmic proteins (Offer and Knight, 1988), as these proteins can be found in purge

loss at approximately 112 mg of protein per milliliter of purge fluid (Savage *et al*, 1990). Cooking losses however contain mainly water (Heymann *et al*., 1990), contain less soluble protein than purge and unlike purge contain fat (Cheng *et al.,* 2008). Water loss of cooked meat is in part due to the vapors generated during the cooking process (Aberle *et al*., 2001), denaturation of proteins (Cheng and Sun, 2008), shrinking of the collagen fibers (Rao *et al*., 1989), and reduction in sarcomere length (Bouton *et al*., 1975). These structural changes result in the expulsion of water from the myofibers (Lawrie, 1998).

1.6.1.6 Proximate Analyses

The proximate analyses methods are used as alternatives to meat dissections and are also used to analyse parts of the carcass that are hard to separate like intramuscular fat. Proximate analyses usually include moisture, protein, fat, and ash. The procedures to assess each of these chemical components are described by the Association of Official Analytical Chemists (AOAC, 1999). Although this method is highly accurate, special attention has to be given to the sample preparation (collection, mixing, weighing) to maintain accuracy. If the researcher intends to obtain a more detailed chemical profile of lean muscle or fat than simply quantity, such as specific minerals, vitamins, myofibrillar proteins, fatty acids and bound versus free water, then spectrophotometric procedures are required and these processes are very difficult to perform adequately and often expensive (Kauffman, 2012).

1.6.2 New Technologies

In the past decade, new technologies have been developed for meat quality prediction and most notable have been those that use visible (VIS) or near-infra-red (NIR) light reflectance to predict tenderness or toughness (Table 1-1). One of the most well described light technologies is the BeefCam, a module that links to the carcass-describing Computer Vision System, which uses reflectance within the optical visual spectrum to sort tough beef (Bowling *et al*. 2009). Another product is the Field Spec Pro Jr., which is capable of sorting beef by tenderness (Rust *et al*. 2008) and works on wavelengths from 400 to 2500 nm. A prototype of a new product called ''The Goldfinch'' is being developed for commercialization and this apparatus works based on NIR hyperspectral images scanned on wavelengths from 965 to 1625nm.

1.6.2.1 Near-Infrared Spectroscopy

In the last decade, VIS and NIR spectroscopy technology have gained popularity in the meat science research community for in-line analysis of meat quality. Light spectroscopy technology is attractive to the meat industry because it is non-invasive, relatively inexpensive, fast, and highly accurate. In past studies, NIR has demonstrated its capability to predict crude protein, intramuscular fat and moisture on beef (Prevolink *et al*., 2005; Prieto *et al*., 2006; Togersen *et al*., 2003), showing a ranging of accuracy in predicting beef toughness from 67 to 93% using recent NIR instrumentation (Cluff *et al*., 2008; Rust *et al*. 2008). Study results from Leroy *et al.* (2003) and Xia *et al.* (2007) suggested that inclusion of visible light to the NIR improved meat quality prediction. Another study showed

that the use of polarization enhances the data obtained from reflectance reading on meat structural profile (Luc *et al*., 2008). According to Swatland, (1989) muscle fibre direction can significantly affect the amplitude of light transmitted through beef, and suggested that inclusion of a directional parameter may improve correlations of light spectroscopy with measurements of meat toughness.

1.7 BEEF GRADING SYSTEMS IN THE WORLD

Because the domestic market for beef is anticipated to continue to decline, export markets for beef will be increasingly important in order to maintain price stability for Canadian producers. Classification and grading of a product is indispensible to setting its value in the global market and is also an important indicator of desired characteristics of a product. The primary purpose of meat description systems is to facilitate trade by describing commercially important attributes (Price, 1995).

Many different carcass assessment and classification systems exist throughout the world (Table 1-2). The United States Department of Agriculture (USDA) Grading System is the most well-known carcass grading system in the world and it currently classifies carcasses into eight beef quality grades applicable to steer and heifer carcasses (Prime, Choice, Select, Standard, Commercial, Utility, Cutter and Canner) and five yield grades designated by number from 1 to 5 (Smith *et al.,* 2008). The Japanese Meat Grading Association uses a similar combination of quality grades numbered 1 to 5 in combination with three yield grades of A through C. In these systems a common national standard is adopted with an assumed quality hierarchy. In Korea, beef carcass grade specifications introduced

by the Korean government in 1992 include quality and yield grades, with the former having 5 levels and the latter 3 categories, resulting in a total of 15 possible categories (Kim *et al.,* 2003). The major function of the quality grades was to segment carcasses into groups that reflected differences in consumer preferences (Polkinghorne and Thompson, 2010). The European system ''EUROP'' main purpose is yield estimation without eating quality connotations (Polkinghorne and Thompson, 2010). The EUROP system is based on a table with carcass conformation characteristics on one axis and external fat level on the other. The carcass conformation axis is divided into five groups; E (extremely muscled), U, R, O and P (very poorly muscled). The U, O and P classes are subdivided into upper (+) and lower (−) sub-groups and in some cases a 15 point conformation scale is used by creating three subdivisions (high, average and low) within each of the EUROP classes. The fat axis is divided in five classes; 1 (very lean) to 5 (very fat). Classes 4 and 5 are divided into lean (L) and fat (H) groups and these are also subdivided into fifteen sub-groups by creating three subdivisions within each fat class (Anonymous, 2008).

One of the most complex and well-structured grading systems is the AUS-MEAT language adopted by the Australian industry in 1987, which uses standardized classification terms to allow individual buyers and sellers to set specific standards (Anonymous, 2006a). The base AUS-MEAT language components (sex, dentition, carcass weight and rib fat) were later complemented by optional evaluation in the chiller of marbling, fat colour, meat colour and carcass ossification To assist with prediction and guarantee of beef tenderness, further pre-slaughter inputs including *Bos indicus* content and hormone growth promotants (HGP) use were

added to the AUS-MEAT assessment system as part of the Meat Standards Australia (MSA) program. The Australian system also incorporates processing factors such as of carcass suspension method (Tenderstretch™), *post mortem* muscle pH and temperature decline, *post mortem* days ageing and anticipated cooking method to predict tenderness by muscle type (Polkinghorne *et al*., 2008). The same muscle could consequently have a different grade depending on the date of consumption or method of cooking. This is defined as a consumer grading system (Polkinghorne and Thompson, 2010).

The South African system classifies carcasses into four age groups based on their dentition: A (no permanent incisors); AB (1 to 2 permanent incisors); B (1 to 6 permanent incisors); and C (more than six permanent incisors). Bulls in age category B or C or castrated males that show signs of late castration are classified and denoted as "MD" to indicate where these carcasses are coming from and indicate that the meat quality (colour, flavor) may be different than those from other carcasses. Fat is evaluated and classified into seven groups that range from 0 (no visible fat) to 6 (excessively fat). Once fat is classified, the resulting number is incorporated into a table that contains fat group scores on one axis and age group scores on the other axis and the result of this combination is a code colour that is later applied as a colored roller brand to carcasses after classification. These colours (purple for A, green for AB, brown for B, red for C and black for the "MD" code) represent the age classification. Five numerical conformation classes (1 (very flat), 2 (flat), 3 (medium), 4 (round) and 5 (very round)) are also designated together with three damage codes; 1 (slight), 2 (moderate) and 3 (serious) where applicable

(Anonymous, 2006b). In South America (Brazil, Argentina, Uruguay and Chile) every country has slightly different beef carcass grading systems, nevertheless, all have common characteristics in that within sex categories, carcasses are classified on maturity and yield with varying emphasis on quality traits (Otaño, 2009). Although a number of South American countries have official grading systems, these are not always used, with many of the large meat companies having their own in-house classification systems that they find more closely aligned with the specifications in the importing countries than the official system (Felico, 2008). In other cases the company uses their own in-house grading systems to benchmark their own brands such as Certified Angus Beef, Hereford Prime, or major retailer house brands rather than relying on generic grading systems (Polkinghorne and Thompson, 2010).

1.7.1 The Canadian Beef Grading System

The Canadian beef grading system began in 1929 and underwent major revisions in 1972, 1992, 1996 and 2001 to increase the accuracy of beef carcass quality and yield assessment and to align it closely to the USA grading system to facilitate marketing. The Canadian beef grading system was designed to assure quality to the customers and to set a carcass value based on carcass quality characteristics or grade standards. These characteristics are directly related to the juiciness, tenderness, consumer acceptability and yield of the carcass. Canadian beef grade criteria are determined by the federal government based on recommendations of its consultative committee, which consists of industry and government meat specialists (Anonymous, 2012d).

The Canadian beef grading system accounts for both carcass yield (yield grade) and meat quality (quality grade), with quality grades estimated based on maturity (age), muscling, masculinity, colour and texture of fat, colour and texture of lean and marbling.

1.7.1.1 Intramuscular Fat (Marbling)

Marbling is the term used to name the intramuscular fat, although this only applies to the flecks of fat deposits in the muscle (Wood *et al* 2008). These flecks of marbling are located in the perimysial connective tissue between muscle fibre bundles and when cooked they may help to separate the muscle fibre bundles, making meat easy to break down in the mouth during the chewing process (Wood, 1990). Marbling content in beef *m. longissimus dorsi* varies along the muscle (Zembayashi and Lunt, 1995) making it difficult to be predicted. Previous studies characterizing the technological properties and eating quality of beef from various degrees or marbling have shown that the degree of marbling has no effect on tenderness (Jones *et al*., 1991). Cole *et al*. (1958) reported that flavor was more important than juiciness and Kropf *et al*. (1959) reported on their study the interrelationship between marbling and subcutaneous fat with flavor. They found that when fat levels increase, the flavor desirability also increased.

Beef carcass marbling is measured by evaluating the average amount, size and distribution of intramuscular fat in the rib eye area. The Canadian beef grading system utilizes four (A – trace, AA – slight, AAA – small, Prime- slightly abundant) of nine recognized levels of marbling by

the USDA (traces, slight, small, modest, moderate, slightly abundant, abundant, moderately abundant and very abundant) (Anonymous, 2012e).

In a Canadian study by Jeremiah *et al*. (1992) consumer preferences for marbling were analyzed and the researchers found that consumer preferences for marbling differed between states in the United States and province to province in Canada. This research, although is more than 20 years old, clearly indicated that consumer preferences change geographically and also most likely demographically. Because United States is Canada' main market, following the meat flow to its final destination is important in order to appropriately commercialize the different Canada grades and their different marbling levels at top price.

1.7.1.2 The Canadian Quality Grades

The Canadian beef grading system is composed of thirteen beef grades. Specifically, in descending order of quality, the Canada beef grades are: Canada Prime, Canada AAA, Canada AA, Canada A, Canada B1, Canada B2, Canada B3, Canada B4, Canada D1, Canada D2, Canada D3, Canada D4, and Canada E and the specific differences between the grades are highlighted in Table 1-3. The highest quality Canadian beef grades are the ''Canada A'' grades (Prime/AAA/AA/A), and these grades are reserved for ''youthful'' (under 30 months of age) carcasses, which represented 87.75% of all graded beef in 2011. The four Canada B grades are also for youthful carcasses that do not meet the minimum quality requirements of the Canada A grades and they represented 1.35 % of all beef carcasses graded in 2011. The four Canada D grades are basically the ''cow grades'' and provide carcass description for cattle older than 30

months, which represented 10.08% of the total graded carcasses in 2011. The E grade is reserved for mature or youthful bull carcasses showing pronounced masculinity and bulls represented 0.8% of the graded carcasses in 2011. A total of 12.79% of Canada's total beef carcasses were not graded in 2011. It is important to mention that almost half of cow and bull carcasses produced in Canada tend not to be graded, and this emphasizes the voluntary aspect of the Canadian beef grading system and suggests that there is no economic advantage to grading carcasses from these cattle. Carcasses that are not graded are referred to as "no-roll" or "ungraded" beef when carcasses are sold. Although the Canadian beef grading system is voluntary, in 2011 87% of the 3 million carcasses were graded in federally/ provincially inspected packing plants (Anonymous, 2012d).

1.7.1.3 The Canadian Beef Grading Agency

The Canadian Beef Grading Agency is a private not-for-profit organization accredited by the Canadian Food Inspection Agency to provide beef grading services in Canada. This corporation has the mission of classifying carcasses into well-defined quality groups and based on this classification assist with establishing a market description for this commodity to facilitate its marketing. Personnel accredited by the Canadian Beef Grading Agency are referred to as graders, and graders evaluate carcasses once they have been chilled (24 to 48 hours after slaughter) to determine carcass muscular development, colour and texture of fat, colour and texture of lean, calculate yield and score the level of marbling in the lean muscle located between the 12th and the 13th rib of

the carcass side. Once these evaluations are made, the carcass is stamped with the corresponding grade and yield.

1.8 SUMMARY

Having reviewed the beef cattle industry inventories and consumer trends in Canadian markets, despite beef decreasing consumption in Canada, beef cattle production and processing continue to be vital economic activities for Albertans with a $12.8 billion turnover due its economic multiplier effect of 4 to 1. To maintain these activities, export markets will continue to be of the utmost importance, and additional effort will be needed to maintain these markets through product differentiation. Continuing the assessment of Canadian beef in local and international markets is essential to having a competitive meat industry in a globalized world, and this can be achieved by investing in developing and improving our beef cattle producing practices as well as putting to work the results of research that has already been done.

Although the Canadian grading system is used to set the value of the whole carcass, the true eating quality profile of each grade has not been determined, nor have the eating quality differences among Canada grades been described in recent years. Prediction of carcasses beef tenderness at the abattoir level is required to ensure that meat is distributed to an appropriate market (Jackman *et al*., 2008) and to determine an appropriate selling price.

To address these concerns, this thesis will address two hypotheses:

That the Canadian beef grading system does not adequately differentiate the quality of the *m. longissimus thoracis* (rib eye muscle);

Quality of the m. *longissimus thoracis* can be predicted from its early *post mortem* muscle conditions (muscle pH and temperature) and *post rigor* colour.

Improving beef tenderness has become one of the main goals of the Canadian beef industry, universities and government research institutions. Examination and understanding the extent to which early *post mortem* and *post mortem* carcass handling practices (chilling temperature, ultimate pH, electrical stimulation and beef ageing) can affect the quality of beef is vital to improving beef quality. In this thesis, the differences in beef quality due to grade were analyzed to determine if the grading system effectively segregated the rib eye muscle (*m. longissimus thoracis*) by its quality. Beef quality in this thesis will be defined by it colour, pH, water holding capacity, cook loss and shear force values as well as fresh meat composition (crude fat, protein and water). We also examined the effect of muscle temperature and electrical stimulation on beef quality, as these practices were the most plausible way to affect *post mortem* metabolism and in consequence alter the colour, texture, and related protein functionality of fresh meats. With this research, understanding of how the Canada beef grading system contributes to beef tenderness was furthered in order to improve the utilization and marketing of these grades.

1.9 TABLES

Table 1-1 Current optical technology for sorting tough and/or tender beef

Name	Accuracy	Wavelength regions	Inconveniences	Reference
BeefCam	64%	Computer Vision System.	Lack of accuracy	Vote *et al*. (2003)
Field Spectro Jr.	92.9%	400 to 2500 nm	Heavy, unwieldy and invasive.	Rust *et al*. (2008)
The Goldfinch	77%	965 to 1625 nm	Prototype	Naganathan *et al*. (2008).

Table 1-2 Principal characteristics of beef classification and grading systems in selected countries (Modified table from Polkinghorne and Thompson, 2010).

	Country							
	Canada	**Europe**	**Japan**	**South Korea**	**The Republic of South Africa**	**United States of America**	**Australia**	
System	Canada	EUROP	JMGA	Korea	South Africa	USDA	AUS-MEAT	Meat Standards Australia
Grading unit	Carcass	Carcass	Carcass	Carcass	Carcass	Carcass	Carcass	Cut
Classification	Yes	Yes	-	–	Yes	–	Yes	–
Quality grade	Yes (4) + (9)	-	Yes (5)	Yes (5)	-	Yes (8)	–	Yes (3)
Yield grade	Yes (3)	–	Yes (3)	Yes (3)	-	Yes (5)	-	-
Pre slaughter	-	-	-	-	-	-	-Grain-fed	-*Bos indicus* % -HGP implants
Slaughter floor	-Carcass weight -Sex -Conformation	-Carcass weight -Sex -Conformation -Fat cover	-Carcass weight -Sex	-Carcass weight -Sex	-Carcass weight -sex -Dentition -Rib fat	-Carcass weight -sex	-Carcass weight -sex -Dentition -Butt shape -P8 site fat depth	-Carcass weight -sex -Electrical stimulation -Hanging method
Chiller	-Marbling score -Meat colour -Meat texture -Fat colour -Fat thickness -Rib-eye muscle area	-	-Marbling score -Meat colour -Meat brightness -Fat colour -Fat luster -Fat texture -Fat firmness -Rib thickness -Fat thickness -Rib-eye muscle area	-Marbling score -Meat colour -Meat texture -Fat colour -Lean maturity -Fat firmness -Fat thickness -Rib-eye muscle area	-	-Marbling score -Meat colour -Ossification score -Meat texture -Rib fat -Kidney and perineal fat -Rib-eye muscle area	-Marbling score -Meat colour -Fat colour	-Marbling score -Meat colour -Ossification score -Hump height -Ultimate pH
Post Chiller	-	-	-	-	-	-	-	-Ageing time -Cooking method

Table 1-3 The 13 Canadian beef quality grades and its requirements

(Table modified from: Anonymous, [2012e])

Grade	Maturity (Age)	Muscling	Rib Eye Muscle	Marbling	Fat Colour and Texture	Fat Measure
CANADA Prime, AAA, AA, A	Youthful	Good to excellent with some deficiencies	Firm, bright red	Prime- Slightly abundant AAA – small AA - slight A - trace	Firm, white or amber	2 mm ≥
B1	Youthful	Good to excellent with some deficiencies	Firm, bright red	No requirement	Firm, white or amber	≤ 2 mm
B2	Youthful	Deficient to excellent	Bright red	No requirement	Yellow	No requirement
B3	Youthful	Deficient to good	Bright red	No requirement	White or amber	No requirement
B4	Youthful	Deficient to excellent	Dark red	No requirement	No requirement	No requirement
D1	Mature	Excellent	No requirement	No requirement	Firm, white or amber	≤ 15 mm
D2	Mature	Medium to excellent	No requirement	No requirement	White to yellow	≤ 15 mm
D3	Mature	Deficient	No requirement	No requirement	No requirement	≤ 15 mm
D4	Mature	Deficient to excellent	No requirement	No requirement	No requirement	15 mm ≥
E	Youthful or mature	Pronounced masculinity				

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Chapter 2

2.0 Bovine *M. Longissimus Thoracis* Quality Differences due to Canada Quality Grade

2.1 INTRODUCTION

Meat quality is the conjunct of acceptable sensory characteristics that meat should have in order to be commercial and profitable. These sensory characteristics are defined by colour, tenderness, juiciness, flavor and aroma (Renerre, 1982; Madruga *et al*., 2010). In Canada, beef under thirty months of age that meets texture, muscling, fat and lean colour requirements is classified into four quality grades based on the amount of intramuscular fat in the *longissimus thoracis* (LT; Rib eye) muscle surface at the 12th and 13th rib interface. The high quality Canada grades are; Canada A (traces of marbling), AA (slight marbling), AAA (small marbling) and Prime (slightly abundant marbling) (Anonymous, 2007). Beef carcass grading allows for an estimation of the eating quality of beef and as a result this classification is used to set the value of the carcass as well. Although the Canadian grading system is used to set the value of the whole carcass, neither the true eating quality profile of each grade nor the eating quality differences among Canada grades been described in recent years. The most recent comparison of beef quality properties by beef grade was in 1976 by Hawrysh *et al.,* while the effects of marbling level on cooking and palatability properties of beef rib-eye steaks was described by

Jones *et al.* (1991). In a study by Lyford *et al.* (2010) it was demonstrated that consumers are willing to pay additional money for increased beef eating quality especially in the Japanese market in a study that included Japan, The United States, Australia and Ireland. Also, in the same study, consumers were willing to pay 1.7 and 2.9 times additional money for 4 and 5 star quality respectively, when 3 stars represented the ''good everyday'' quality, while consumers were willing to pay only half price for the 2 stars product. Several studies have reported that meat tenderness is the most important quality trait for the consumer (Verbeke *et al*., 2010; Savell *et al*., 1987) and have shown that consumers are willing to pay an increased price for guaranteed tender meat (Platter *et al*., 2005; Miller *et al*., 2001). Understanding which meat quality characteristics contribute to and affect tenderness is important, particularly the influence of intramuscular fat (marbling) because marbling score is the only parameter used to differentiate between the Canada quality grades. Therefore, the objectives of this study were to characterize the meat quality differences between the LT from the four quality Canada grades and the relationship between fat content and tenderness. The results of this study have the final goal of understanding Canadian beef based on its meat quality profile to target appropriate local and international markets.

2.2 MATERIALS AND METHODS

2.2.1 Experimental Design

Twelve LT (rib eye) muscles from each quality grade (Canada A, AA, AAA & Prime) were obtained from a large Alberta abattoir within 3

days *post mortem* over four shipments. Animal genetics and physical characteristics were not known as the muscles were purchased from the abattoir. Certified meat graders by the Canadian Beef Grading Agency determined the quality grade of each sample within 24 h *post mortem* by viewing the surface of the *m. longissimus thoracis* muscle between the 12th and 13th rib (posterior part of the muscle). A total of 48 LT muscles were accepted into the study and muscles were divided into anterior and posterior for analysis, with the posterior portion analyzed at 3 days aging and the anterior portion analyzed after 14 days aging. The portions were not randomized in order to meet the requirements of another study and this is an acceptable practice in beef aging studies (Mehaffey *et al*., 2009). From the posterior of each muscle portion, four 2.5 cm thick steaks were removed, with the first steak used for objective colour measurements, the second used proximate analyses, the third for cooking loss and Warner–Bratzler shear force (WBSF) and the fourth one for pH and drip loss. Measurements at 14 days were performed on the anterior portion of the LT; where three steaks of 2.5 cm each were removed from the posterior of the portion. The first steak was used for objective colour measurements, the second one was used for cooking loss and Warner–Bratzler shear force and the third one was used for pH and drip loss.

2.2.2 Colour Analysis

Colour analysis is improved by increasing the number of measurements within a sample (Alcalde *et al.,* 2001); therefore, 3 locations along the LT muscle surface were chosen to take measurements. The three values were averaged and the mean value recorded. Prior to colour

analysis, approximately 3 mm of the outside face was removed and the newly exposed surface was allowed to bloom (oxygenation) in a tray covered with oxygen-permeable polyvinyl-chloride film (Fisher Scientific, Pittsburgh, PA, USA.) at 4 °C for 60 min. Lightness (L^*), green-red (a^*) and blue–yellow (b^*) were determined with a Minolta Chromameter CR-400 (Konica-Minolta, Osaka, Japan) using the colour system establish by the Commission Internationale de L'Eclairage (CIE; CIE, 1976). The instrument was calibrated against a white tile provided with the instrument before use.

2.2.3 Water Holding Capacity, Cooking Loss and Cook Time

The water holding capacity was determined by drip loss as described by Honikel (1998) and as outlined by the American Meat Science Association methods (AMSA, 1995). Drip loss was measured by first trimming the external fat from the LT steak where weights ranged from 106 – 200 g (Table 2-1). Steaks were hung for 24 hours at 4 °C in a plastic bag from a stainless steel hook. The weight of the trimmed steak portion was recorded before and after the procedure, and water loss results expressed as a percentage of the original weight. To calculate cooking loss, additional LT steaks were trimmed of subcutaneous fat, weighed and grilled on a pre-heated grill (General Electric 4 in 1 Grill / Griddle, China) set to a temperature of 176 °C. The internal temperature of each steak was monitored continuously using a stainless steel thermocouple attached to a recording device (Tinytag View 2s, manufacturer, city) inserted into the geometric centre of the steak. Steaks were heated until the steak internal temperature reached 71 °C. Once an internal temperature of 71 °C was

reached, the cooked steaks were cooled to less than 10 °C in an ice bath and then weighed the next day after cooling. Cooking loss was calculated by dividing the steak weight loss during cooking by the trimmed raw weight of the steak and reported as a percentage of the initial raw weight. Cooking time was recorded every 3 minutes until steak internal temperature reached 71 °C using a four-channel alarm timer (Traceable ®, Fisher Scientific, Edmonton, CA.), and cook time was calculated as minutes/100 g of fresh product.

2.2.4 Warner-Bratzler Shear Force

Cooked steaks were stored overnight at 4 °C, and the following day steaks were removed from refrigerated storage and allowed to reach room temperature. Once the cooked steaks reached room temperature, 6 cores of 1.27 cm diameter and 2 cm long were removed from each steak parallel to the muscle fibers using a cork borer. Each core was sheared once across the middle, perpendicular to the fiber direction, using a materials testing machine (AMETEK, Inc. Lloyd Instrument LRX plus, Digital Metrology Measurements, Kitchener, ON) fitted with a Warner–Bratzler type shear blade travelling at 225 mm/min. Shear force was expressed in Newtons (N) and values were averaged to obtain a mean value for each steak.

2.2.5 pH

Measurements of pH were performed with a temperature-compensated pH meter (Fisher Scientific, Accumet Waterproof AP71 pH/mV/Temperature, Fisher Scientific, Toronto, ON) fitted with a glass probe electrode (Hanna Instruments, Fisher Scientific, Toronto, ON) which

was inserted into the muscle. Three readings were taken from each muscle and the pH values were averaged and the mean used for statistical analysis. Prior to pH measurement, the pH meter and electrode were standardized using pH buffers of 4.0 and 7.0 at room temperature (23 °C).

2.2.6 Proximate Analyses

To determine the chemical composition of the beef samples, approximately 100 g of minced sample were weighed and placed into an aluminium tray and lyophilized for four days using a freeze dryer (Virtis freeze dryer ultra El-85, SP Scientific, Warminster, PA., USA.). Upon the completion of lyophilisation, the trays were removed from the freeze dryer and final weights recorded for moisture loss calculation. Crude fat analysis was determined by pulverizing the freeze-dried sample into a fine powder using a blender fitted with a 1 L stainless steel container (Two-Speed Food Blender Model 7011G; Waring Commercial. Torrington, CT., USA) containing two to three pellets of dry ice. Duplicates of two grams of this dry meat powder were placed in thimbles and analyzed for crude fat content (Method 960.39; Association of Official Analytical Chemists 1995) by petroleum ether extraction (Goldfisch Fat Extraction Apparatus Model 35001; Labconco Corp. Houston, TX, USA.) A blank/control sample was run at all times to avoid false results, and duplicates were averaged to complete statistical analysis. Nitrogen content was determined using triplicates of 100 mg of ground dry meat sample (Method 992.15; Association of Official Analytical Chemists 1997) (Nitrogen/Protein Determinator CNS2000, Leco Corp., St., Joseph, MI., USA.) and triplicates were averaged and means used for statistical

analysis. Standardization and calibration of the Leco machine was performed with three replicates of caffeine and ethylenediaminetetraacetic acid (EDTA) samples prior to meat sample analyses.

2.2.7 Statistical Analyses

Data were organized as a randomized complete block design and analyzed using the MIXED procedure in the Statistical Analysis System (SAS) software (Version 9.2, Statistical Analysis Systems, Cary, NC, USA). Analysis of variance was conducted using beef grade as the sole fixed effect. Ribs were blocked by replicate of grades and block was included as a random source of variation. Data from non-aged (3 days) and aged (14 days) beef were analyzed in separate data sets due to aging treatment being confounded with sample location along the LT. Grade effect was considered significant at $P \leq 0.05$ and where grade was significant least square mean differences were used to determine differences between grade means with significance at $P \leq 0.05$, and least of square means differences were separated using the PDIFF function of SAS. In all analyses of variance, degrees of freedom were corrected using the Kenward-Roger adjustment. Correlations analyses were performed using PROC CORR to obtain Pearson correlations among the meat quality data to calculate possible linear dependence among variables. Correlation analyses were performed on drip and cook loss percentages in relation to steak weight and were used to identify any dependence of drip and cook loss percentage on steak weight.

The randomized complete block design model was:

$yij = \mu + Gi + Bj + \varepsilon ij$

Where μ was the overall mean, G_i was the grade, where i = 1...4, B_j was the block where j = 1...12 and ε_{ij} was the random deviation associated with each observation.

2.3 RESULTS

2.3.1 pH and Colour

Mean ultimate pH value for the 3 day, non-aged samples was statistically greatest in the Canada Prime LT, even though it was higher than that of the LT muscles from the other grades by only 0.2 pH unit (Table 2-1). After 14 days of additional ageing, however, there was no difference in the mean ultimate pH of the LT muscles across the grades (Table 2-2).

Without ageing, LT (rib-eye) steaks from Canada Prime had the highest mean L^* value and were the most yellow (b^*) (Table 2-1). There were no differences in a^* values (coordinate green–red) among grades, although there was a trend toward Prime rib eyes to be the most red (P = 0.073). In samples aged for 14 days, differences in colour were only significant in terms of mean L^* values, with beef from the Canada Prime grade having the highest value (Table 2-2). There was a tendency (P = 0.0611) for LT from the Canada A grade to be the most blue and for that from the Canada Prime grade to be the most yellow.

2.3.2 Drip Loss, Cook Loss and Cook time

Differences in drip loss between grades were limited to trends only (P = 0.057) in the non-aged samples with rib eye muscle from the Canada A

grade tending to have the most drip loss (Table 2-1). This tendency did not persist with additional ageing, with the 14 day aged LT muscles showing no effect of grade on drip loss (Table 2-2).

There were significant differences (P= 0.0017) between the non-aged LT due to grade for cooking loss, with rib muscle from the Canada Prime grade having a lower mean cooking loss value than that of rib muscles from all other grades (Table 2-1), and this difference persisted in the aged samples (P = 0.0204) (Table 2-2).

Differences in cook time between grades were limited to trends only (P = 0.054) in the non-aged samples with rib eye muscle from the Canada Prime grade tending to take longer to be cooked (Table 2-1). This tendency did not persist with additional ageing, with the 14 day aged LT muscles showing no effect of grade on cook time (Table 2-2).

2.3.3 Warner-Bratzler Shear Force

There was no difference in Warner-Bratzler shear force (WBSF) values between the grades in non-aged samples although there was a trend for beef from Canada Prime ribs to be more tender than that from ribs of the other quality grades (P = 0.124) (Table 2-1). Differences in aged samples were found (P = 0.0009) with Canada AA having the highest and Canada AAA and Prime having the lowest WBSF values (Table 2-2).

2.3.4 Proximate analyses

Proximate analyses were performed on non-aged LT muscle. Differences in crude fat content among grades were significant (P < 0.0001),

with the Canada Prime grade LT muscle having the highest mean crude fat content (11.57%), followed by Canada AAA LT (4.62%). The lowest crude fat contents were observed in LT from the Canada AA (3.11%) and A grades (2.92%), the means of which were not significant different from each other (Table 2-3).

Differences in moisture content were significant ($P < 0.0001$) with Canada Prime having the lowest moisture content (64.64%), which was statistically different from the mean moisture contents of LT muscles from the Canada AAA, AA and A grades. The mean percentage moisture content values of LT from the latter three grades were not different from each other (Table 2-3).

Protein content was significantly affected by grade ($P < 0.0001$), with LT from the Canada A and AA having the highest mean protein contents and the LT from the Canada AAA and Canada Prime having the lowest protein contents (Table 2-3).

2.3.5 Correlations

2.3.5.1 Drip and cook loss correlation to steak weight

There was no significant correlation between the weights of steak-portions used for drip loss and the actual percentage of drip loss ($P = 0.9340$) (Table 2-4). There was, however, a trend ($P = 0.0937$) toward a linear correlation between steak weight and cook loss (Table 2-5).

2.3.5.2 Correlation among meat quality traits of beef m. longissimus thoracis

At 3 days, significant correlations were found and are reported in Table 2-6. L* was correlated to b* (r = 0.39), cook time (r = 0.30), crude fat (r = 0.51), moisture (r = -0.45) and protein content (r = -0.31). For other colour coordinates, a* was correlated to b* (r = 0.41) and Warner-Bratzler shear force (r = -0.50). In addition, b* was correlated to drip loss (r = -0.34), cook loss (r = -0.29), crude fat (r = 0.40) and protein (r = -0.50). Intramuscular pH was correlated to drip loss (r = -0.40), cook loss (r = -0.35), crude fat (r = 0.49) and protein content (r = -0.42). Drip loss was correlated to protein content (r = 0.32). Cook Loss was correlated to Warner-Bratzler shear force (r = 0.38), crude fat (-0.41), moisture (r = 0.29) and protein content (r = 0.43). Warner-Bratzler shear force was correlated to cook time (r = 0.33), crude fat (r = -0.36), moisture (r = 0.36) and protein contents (r = 0.29). Cook time was correlated to crude fat (r = 0.32) and moisture (r = -0.37). Crude fat was correlated to moisture (r = -0.84) and protein contents (r = -0.65). Moisture was correlated to protein (r = 0.35).

At 14 days significant correlations were found and are reported in Table 2-7. L* was correlated to b* (r = 0.61) and crude fat (r = 0.32) while a* was correlated to b* (r = 0.66), pH (r = -0.36) and Warner-Bratzler shear force (r = -0.45). Also, b* was correlated to pH (r = -0.28), drip loss (r = -0.29), cook loss (r = 0.28), and protein content (r = -0.29). Intramuscular pH was correlated to crude fat (r = 0.35). Drip loss was correlated to protein content (r = 0.29). Cook Loss was correlated to cook time (r = 0.48), crude fat (-0.29) and moisture content (r = 0.41). Warner-Bratzler shear force was correlated to and protein (r = 0.32). Cook time was correlated to protein (r =

-0.37). Crude fat was correlated to moisture (r = -0.84) and protein (r = -0.65). Moisture was correlated to protein (r = 0.35).

2.4 DISCUSSION

Previous studies characterizing the technological properties and eating quality of beef from various degrees or marbling have shown that the degree of marbling has no effect on tenderness (Jones *et al*., 1991) as discussed in Chapter 1 section 1.7.1.2 of this thesis. The Canadian high quality grades are sorted by degree of marbling (marbling score) therefore study of the effect of marbling on not only tenderness but colour as well is important because colour is one of the most important factors to influence customers during purchasing of meat. This is because customers relate a bright cherry red colour with freshness and a brownish or pale colour could result in rejection of the product (Hood and Riordan, 1973).

Regular characterization of beef from each quality grade is warranted because cattle genetics change with time and market selection pressures. The current study showed that without ageing, beef from the Canada Prime grade was different from that of the other Canada quality grades in terms of its intramuscular pH, luminosity and colour (b*). Lean *L** values at 3 and 14 days may have been increased in the Prime grade by the large amount of intramuscular fat related to this grade, as increased lightness of lean has been related to warm muscle temperatures early *post mortem* (Bruce and Ball, 1990). Warm muscle temperatures early *post mortem* could arise from increased subcutaneous fat, which can be associated with carcasses that have high levels of marbling (Moon *et al*., 2006). A high early *post mortem* temperature can also denature muscle

proteins, which could increase colour reflectance either through structural alteration of the myofibrillar proteins (Sleper *et al*., 1983) or increased water exudation (Scopes, 1964; Scopes, 1970).

Previous studies have shown that meat colour darkens as intramuscular pH increases (Abril *et al*., 2001). In the present study, Prime rib eye muscle had the highest mean pH, but it was only 0.1 pH value greater than that of the other grades (Table 2-4). This increase in pH did not affect the colour of the lean as the lean of rib eye muscles from the Canada A were not as red and bright than those of Canada Prime because they had decreased mean *a** and *b** values (Table 2-4). The lean of Canada A and AA LT muscles may be darker than that of Prime beef due to an increase in the oxygen consumption rate by *post rigor* beef muscle. The rate of oxygen consumption by beef is decreased by high early *post mortem* muscle temperature because inner mitochondrial membrane proteins and myoglobin are denatured (Young *et al*., 2001), and this contributes to an attractive bright red appearance of the exposed meat surface (Bendall and Taylor, 1972).

Pearson correlation results confirmed that colour is an important characteristic of beef that is related to beef eating quality. The a* value was able to explain approximately 50 % of the variation in terms of Warner-Bratzler shear force values, while the L* explained 51% of the variation in crude fat content. Measurements of L* could have been affected by marbling, due that in some instances avoiding marbling is a very difficult task. Therefore, when measuring the colour of highly marbled meat, special attention should be given to avoiding marbling deposits.

Data from b* measurements explained 50% of the variation in protein content at 3 days (Table 2-7). At 14 days ageing, however, the colour correlation of b* with protein content decreased considerably (r = -0.29) and the correlation between L* and protein content disappeared. The ability of the a* value to predict Warner-Bratzler shear force also decreased with ageing from 50% to 45% (Table 2-7) but maintained its significance. In a study by Wulf *et al* (1997) where colour was measured at 27 hours *post mortem*, correlations between shear force and L*, a* and b* values were also found and were -0.36, -0.24 and -0.38 respectively. The study of Wulf *et al*. (1997) most likely had significant linear relationships between shear force and all colour coordinates because of the diversity of its experimental design, which used *Bos taurus* and *Bos indicus* heifers and steers, giving the experiment a larger amount of variation than the present study. This is something that should be considered if an algorithm for predicting shear force of meat is desired as different feeding programs or *post mortem* carcass treatments such as chilling temperatures (Bruce and Ball, 1990) and electrical stimulation (Li *et al*., 2011) can affect beef colour stability.

Unexpectedly, there was no difference in mean shear force values due to Canada grade at 3 days ageing. This was unexpected because the difference in premium (average) between Canada Prime and Canada AAA can be as much as +$10/cwt (hundred weight or centum weight) for carcasses graded Canada Prime (Jim, 2012). Although not statistically significant at P = 0.124, the differences in peak shear force between the four grades at 3 days (non-aged) could become significant if 24 rather than 12 replicates were included in the study. A power analysis based upon the mean and the standard error observed for the dependent variables in

the present study indicated that doubling the sample size would increase the statistical power of the experiment from 0.48 to 0.82 (Table 2-8) and would decrease the likelihood of a Type II error.

It was observed that mean shear force values at 14 days had become unexpectedly tougher than those from 3 days, when literature shows that meat tenderizes over time after animal exsanguination (Koohmaraie *et al.,* 1987). Shear force values in this case may have been affected by the cooking method as stated by Obuz *et al* (2004), or by the cooking process with grilling machines malfunctioning or cooking at different rates. Steak location within the muscle may have affected tenderness as well, because 14 days samples were collected 5 inches from the graded face (12^{th} and 13^{th} rib interface) of the rib eye, and literature shows that rib eye steaks located closer to the chuck are tougher than those from the graded site (Janz *et al.,* 2006). Therefore randomization of samples is warranted when comparing ageing treatments within the same muscle despite the existence of studies in which this is not considered (Mehaffey *et al*., 2009).

Despite the increase in toughness observed in the Canada AA rib sections, the Canada Prime LT steaks were more tender than those from Canada AA and the Canada AAA steaks were more tender than LT from Canada A and AA after 14 days aging. This suggested that the aging capacity for Canada AAA and Prime beef was greater than that of the A and AA grades. In light of this, the LT within the Canada AAA and Prime are appropriately valued higher than that from the Canada A and AA grades.

As expected, there were differences in crude fat content among the Canada high quality grades observed in this study, especially the

difference between Canada Prime with the other grades. Crude fat content was negatively correlated (r= -0.36) with Warner-Bratzler shear force at 3 day study, but, this correlation disappeared in the 14 day study. These findings agree with those of Jones *et al*., (1991) where marbling level had no influence on overall beef tenderness.

2.5 CONCLUSION

This study indicated that LT muscles from Canada Prime and AAA had meat quality characteristics different from those of the other grades, suggesting that Prime and AAA LT are appropriately valued as superior products. According to the crude fat analysis of the Canada quality grades, Canada A and AA had the lowest fat content of all four grades; therefore, the beef industry could better market these grades within consumer populations that express interest in low fat content meat products. The lack of meat quality measurement differences among LT muscles graded Canada A, AA and AAA suggested that LT from A and AA may be undervalued, but the low statistical power of the experiment dictates that this conclusion be viewed with caution. Further research on the quality grades is needed to adequately describe the eating quality of the beef from these grades so that a complete quality profile of Canadian beef can be achieved.

2.6 TABLES

Table 2-1 Meat quality of high quality Canada grades of 3 days samples

	NON -AGED CANADA GRADES					
Analysis	A	AA	AAA	PRIME	Pr > F[1]	SEM[2]
n	12	12	12	12		
L*	36.3[a]	36.8[a]	35.9[a]	39.4[b]	0.0006	0.88
a*	18.5	19.2	20.3	21.3	0.0734	1.15
b*	2.9[a]	3.6[a]	3.6[a]	5.9[b]	0.0022	0.80
pH	5.3[a]	5.3[a]	5.4[ab]	5.5[b]	0.0494	0.04
Drip Loss (%)	0.8	0.7	0.6	0.5	0.0567	*0.29*[*]
Cook Loss (%)	17.2[a]	18.7[a]	15.9[ab]	14.6[b]	0.0017	0.70
Cook Time (min/100gr)	2.2[a]	2.4[ab]	2.4[ab]	2.6[b]	0.054	0.12
WBSF[3] (Newton)	42.6	44.9	45.0	32.2	0.1240	4.28

[1] Probability of the calculated F value with significance at $P \leq 0.05$

[2] SEM - Standard error of the mean

[a, b] Means with different superscripts within a row are significantly different at $P < 0.05$ according to least square mean differences tests.

[3] WBSF – Warner-Bratzler Shear Force

[*]Standard Deviation

Table 2-2 Meat quality of high quality Canada grades of 14 days samples

	AGED CANADA GRADES					
Analysis	A	AA	AAA	PRIME	Pr > F[1]	SEM[2]
n	12	12	12	12		
L*	33.2[a]	32.6[a]	31.8[a]	35.7[b]	<0.0001	1.1
a*	19.1	20.9	21.0	21.1	0.4664	1.5
b*	1.2	2.6	1.8	3.1	0.0611	.73
pH	5.5	5.5	5.5	5.6	0.3731	0.03
Drip Loss (%)	0.81	0.72	0.72	0.69	0.1906	*0.14*[*]
Cook loss (%)	15.5[a]	16.1[a]	14.0[ab]	12.7[b]	0.0204	1.1
Cook Time (min/100gr)	2.1	2.0	2.1	2.1	0.66	0.12
WBSF[3] (Newton)	44.1[a]	52.6[b]	35.7[c]	39.6[ac]	0.0009	5.0

[1] Probability of the calculated F value with significance at $P \leq 0.05$

[2] SEM - Standard error of the mean

[a, b] Means with different superscripts within a row are significantly different at $P < 0.05$ according to least square mean differences tests.

[3] WBSF – Warner-Bratzler Shear Force

[*] Standard Deviation

Table 2-3 Means of proximate analyses from high quality Canada grades

	CANADA GRADES					
Analysis %	A	AA	AAA	PRIME	Pr > F[1]	SEM[2]
n	12	12	12	12		
Crude Fat	2.92 [a]	3.11 [a]	4.62 [b]	11.57 [c]	<.0001	0.4879
Moisture	70.63 [a]	70.69 [a]	69.28 [a]	64.64 [b]	<.0001	0.8850
Protein	24.33 [a]	24.34 [a]	23.56 [b]	22.14 [c]	<.0001	0.3493

[1] Probability of the calculated F value with significance at $P \leq 0.05$

[2] SEM - Standard error of the mean

[a, b, c] Means with different superscripts within a row are significantly different at $P < 0.05$ according to least square mean differences tests.

Table 2-4 Correlation between drip loss factors at 3 and 14 days ageing

Variable	Steak Weight at 0 h	Steak Weight at 24 h	Weight Loss	Drip Loss %
Steak Weight at 0 h	1.00	.99 <.0001	0.47 <.0001	-0.00 0.9340
Steak Weight at 24 h	0.99 <.0001	1.00	0.46 <.0001	-0.02 0.8360
Weight Loss	0.47 <.0001	0.46 <.0001	1.00	0.86 <.0001
Drip Loss %	-0.00 0.9340	-0.02 0.8360	0.86 <.0001	1.00

Table 2-5 Correlation between cook loss factors at 3 and 14 days ageing

Variable	Steak Raw Weight	Steak Trimmed Weight	Steak Cooked Weight	Steak Weight Loss	Cook Loss %
Steak Raw Weight	1.00	.90 <.0001	0.85 <.0001	0.68 <.0001	0.17 0.09
Steak Trimmed Weight	0.90 <.0001	1.00	0.97 <.0001	0.68 <.0001	0.08 0.4007
Steak Cooked Weight	0.85 <.0001	0.97 <.0001	1.00	0.50 <.0001	-0.13 0.1781
Steak Weight Loss	0.68 <.0001	0.68 <.0001	0.50 <.0001	1.00	0.77 <.0001
Cook Loss %	0.17 0.0937	0.08 0.4007	-0.13 0.1781	0.77 <.0001	1.00

Table 2-6 Pearson correlation coefficients among meat quality traits of beef m. *longissimus thoracis* (Rib Eye) at 3 days

Variable	L*	a*	b*	pH	Drip Loss (%)	Cook Loss (%)	WBSF[1] (Newton)	Cook Time (min/100g)	Fat Content (%)	Moisture Content (%)	Protein Content (%)
n	48	48	48	48	48	48	48	48	48	48	48
L*	1.00	-0.05	0.39*	0.27	-0.12	-0.18	-0.09	0.30*	0.51*	-0.45*	-0.31*
a*	-0.05	1.00	0.41*	0.08	0.07	-0.08	-0.50*	0.04	0.27	-0.23	-0.31*
b*	0.39*	0.41*	1.00	0.10	-0.34*	-0.29*	-0.03	0.23	0.40*	-0.21	-0.50*
pH	0.27	0.08	0.10	1.00	-0.40*	-0.35*	-0.14	0.04	0.49*	-0.26	-0.42*
Drip Loss (%)	-0.12	0.07	-0.34*	-0.40*	1.00	0.27	-0.19	-0.06	-0.27	0.12	0.32*
Cook Loss (%)	-0.18	-0.08	-0.29*	-0.35*	0.27	1.00	0.38*	0.11	-0.41*	0.29*	0.43*
WBSF (Newton)	-0.09	-0.50*	-0.03	-0.14	-0.19	0.38*	1.00	0.33*	-0.36*	0.36*	0.29*
Cook Time (min/100g)	0.30*	0.04	0.23	0.04	-0.06	0.11	0.33*	1.00	0.32*	-0.37*	-0.15
Fat Content (%)	0.51*	0.27	0.40*	0.49*	-0.27	-0.41*	-0.36*	0.32*	1.00	-0.84**	-0.65**
Moisture Content (%)	-0.45*	-0.23	-0.21	-0.26	0.12	0.29*	0.36*	-0.37*	-0.84**	1.00	0.35*
Protein Content (%)	-0.31*	-0.27	-0.50*	-0.42*	0.32*	0.43*	0.29*	-0.15	-0.65**	0.35*	1.00

[1]WBSF – Warner-Bratzler Shear Force

* P=<0.05

** P=<0.0001

Table 2-7 Pearson correlation coefficients among meat quality traits of beef m. *longissimus thoracis* (Rib Eye) at 14 days

Variable	L*	a*	b*	pH	Drip Loss (%)	Cook Loss (%)	WBSF[1] (Newton)	Cook Time (min/100g)	Fat Content (%)	Moisture Content (%)	Protein Content (%)
n	48	48	48	48	48	48	48	48	48	48	48
L*	1.00	0.26	0.61**	-0.14	-0.05	0.15	-0.09	0.06	0.32*	-0.17	-0.27
a*	0.26	1.00	0.66**	-0.36*	0.06	0.18	-0.45*	0.06	0.07	-0.05	-0.12
b*	0.66**	0.66**	1.00	-0.28*	-0.29*	0.28*	-0.15	0.26	0.10	0.07	-0.29*
pH	-0.14	-0.36*	-0.28*	1.00	-0.13	-0.12	0.11	-0.02	0.35*	-0.16	-0.15
Drip Loss (%)	-0.05	0.06	-0.29*	-0.13	1.00	0.03	-0.06	-0.15	-0.20	0.02	0.29*
Cook Loss (%)	0.15	0.18	0.28*	-0.12	0.03	1.00	0.22	0.48*	-0.29*	0.41*	0.04
WBSF (Newton)	-0.09	-0.45*	-0.15	0.11	-0.06	0.22	1.00	0.09	-0.19	0.18	0.32*
Cook Time (min/100g)	0.06	0.06	0.26	-0.02	-0.15	0.48*	0.09	1.00	0.06	0.09	-0.37*
Fat Content (%)	0.32*	0.07	0.10	0.35*	-0.20	-0.29*	-0.19	0.06	1.00	-0.84**	-0.65**
Moisture Content (%)	-0.17	-0.05	0.07	-0.16	0.02	0.41*	0.18	0.09	-0.84**	1.00	0.35*
Protein Content (%)	-0.27	-0.12	-0.29*	-0.15	0.29*	0.04	0.32*	-0.37*	-0.65**	0.35*	1.00

[1]WBSF – Warner-Bratzler Shear Force

* P=<0.05

** P=<0.0001

Table 2-8 Power analysis of Warner-Bratzler shear force (WBSF) at 3 days

Power Analysis of Warner-Bratzler Shear Force (WBSF) at 3 days						
Dependent Variable	Source	Alpha	Sample Size	Sample Size Type	Power	Least Significant Number
WBSF	Grade	0.05	48	Observed	0.485	66
WBSF	Grade	0.05	50	Specified	0.504	.
WBSF	Grade	0.05	52	Specified	0.524	.
WBSF	Grade	0.05	54	Specified	0.542	.
WBSF	Grade	0.05	56	Specified	0.560	.
WBSF	Grade	0.05	58	Specified	0.578	.
WBSF	Grade	0.05	60	Specified	0.596	.
WBSF	Grade	0.05	62	Specified	0.612	.
WBSF	Grade	0.05	64	Specified	0.629	.
WBSF	Grade	0.05	66	Specified	0.645	.
WBSF	Grade	0.05	68	Specified	0.660	.
WBSF	Grade	0.05	70	Specified	0.675	.
WBSF	Grade	0.05	72	Specified	0.690	.
WBSF	Grade	0.05	74	Specified	0.704	.
WBSF	Grade	0.05	76	Specified	0.717	.
WBSF	Grade	0.05	78	Specified	0.731	.
WBSF	Grade	0.05	80	Specified	0.743	.
WBSF	Grade	0.05	82	Specified	0.755	.
WBSF	Grade	0.05	84	Specified	0.767	.
WBSF	Grade	0.05	86	Specified	0.778	.
WBSF	Grade	0.05	88	Specified	0.789	.
WBSF	Grade	0.05	90	Specified	0.800	.
WBSF	Grade	0.05	92	Specified	0.810	.
WBSF	Grade	0.05	94	Specified	0.819	.
WBSF	Grade	0.05	96	Specified	0.828	.
WBSF	Grade	0.05	98	Specified	0.837	.

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Chapter 3

3.0 Interaction Effects of Chiller Temperatures, Electrical Stimulation and Ageing on Beef Quality

3.1 INTRODUCTION

The process of applying an electrical current through a carcass before the onset of *rigor mortis* is called electrical stimulation (ES). When applied, electrical stimulation reduces the susceptibility of pre-*rigor* muscle to cold shortening by inducing vigorous muscular contractions throughout the carcass. These contractions promote the use of energy in the form of adenosine triphosphate (ATP) by the muscles and in response to the increased energy expenditure a dramatic acceleration of pH decline follows. Electrical stimulation of a beef carcass usually reduces the muscle pH by 0.5 units with 1 minute of electrical stimulation. A similar decline of pH (0.5 units) in a carcass that does not receive electrical stimulation usually requires three or more hours (Ducastaing *et al*., 1985).

Electrical stimulation of beef carcasses has significant positive effects on lean maturity score, overall maturity score, and Warner–Bratzler shear force values (Calkins *et al*., 1980). A detailed understanding of how electrical stimulation affects meat quality is presented in Chapter 1, Section 1.5.7.

Livestock carcasses are chilled so that food safety is ensured, maximum shelf life maintained and shrinkage of muscles is reduced, with

little focus on maintaining tenderness and colour of the finished product (Savell *et al*., 2005). Hwang and Thompson (2001) indicated that early *post mortem* conditions can determine the toughness and appearance of beef. Although meat tenderness has been improving as shown in the recent 2001-2011 Canadian beef tenderness survey (Juarez *et al.,* 2013), there is still opportunity for improvement as 1% of strip loins, 5% of top sirloin, 13% of inside rounds and 8% of cross rib steaks were found to be tough. Hwang and Thompson (2001) advocated the use of electrical stimulation to control beef quality but it may interact with chiller temperature. Aalhus *et al.* (2001) acknowledged that by combining blast chilling and electrical stimulation, shear force values in the *m. longissimus lumborum* of beef sides were decreased by 9.5% in comparison with conventionally chilled carcass sides. Aalhus *et al.* (2001) also found that blast chilling had no effect on marbling score, and also reported that blast-chilled/electrically stimulated carcasses had brighter lean colour than conventionally chilled carcasses. Therefore understanding how electrical stimulation and different chilling regimes in combination with traditional post mortem ageing is crucial to preserving beef eating quality. The study objectives were to identify advantages or disadvantages of the use of electrical stimulation on beef quality and interactions between chiller temperatures and changes induced by electrical stimulation.

3.2 MATERIALS AND METHODS

3.2.1 Experimental Design

The effects of early *post mortem* carcass temperature, muscle pH and ageing on beef quality measurements were investigated using a split-split-plot design, with two chilling temperatures (warm and cold) in the main plot, two electrical stimulation treatments (control and stimulated) in the sub-plot and two aging treatments (3 and 14 days) in the sub-sub-plot.

3.2.1.1 Animal Selection and Slaughter

Twenty Angus crossbred cattle from the University of Alberta Kinsella Ranch were balanced into two slaughter groups of ten (n=10) based on stratified live weight as heavy carcasses are known to be associated with the reddest meat, the whitest fat and the highest marbling scores (Moon *et al*., 2003). Cattle were shipped by trailer two weeks apart to the Agriculture and Agri-Food Canada (AAFC) Meat Research Centre research abattoir the morning of slaughter and allowed to rest up to two hours. Once at the abattoir, cattle had *ad libitum* access to water and were separated from all other animals. Live weights and animal identification were recorded and animals were stunned, exsanguinated and dressed in a simulated commercial manner in accordance with the ethical principles and guidelines recommended by the Canadian Council on Animal Care (Olfert *et al*., 1993).

3.2.1.2 Carcass Management

Following splitting of the carcass, hot carcass side weights were recorded (trimmed) and electrical stimulation (ES) was applied to right sides at 450 volts for 90 sec (Koch-Britton Stimulator Model 350, Kansas, USA.). Initial (1 hour) pH and temperature values were collected on both the left and right m. *longissimus lumborum* (LL) using a Hanna HI99163 pH meter equipped with a Hanna Smart electrode FC232 for meat (Hanna Instruments, Laval QC). At the same time and location, a muscle core sample (approximately 10 g) was extracted from the carcass using a Black and Decker Cordless Drill (Model HP818 [Sears Canada) with a 37 mm core attachment. The subcutaneous fat cap was removed from the core, and then the core was sample flash frozen in liquid nitrogen, placed in pre-labeled Whirlpak™ (Fisher Scientific, Edmonton, Alberta) bags and stored at -80°C for analysis of glucidic metabolites. Carcass sides were pasteurized using a steam pasteurizer at 85°C for ten seconds and then chilling treatment was applied.

Carcasses (both left and right sides) assigned to the warm chilling treatment (n = 5/kill) were railed into a 2°C chiller with air speeds of 0.5 m/sec, while carcasses (both sides) assigned to the cold chill treatment were railed into a -2°C cooler (TEMP). Upon entry into the respective cooler, stainless steel thermocouples (10 cm) were placed into the right and left loin and hip and temperatures recorded (15 minutes intervals) using data temperature loggers (Mark III, MC4000 [Sumaq Wholesalers, Toronto, ON]). Carcasses railed into the -2 °C cooler had frozen ice-packs attached to the loin area for carcasses while 36°C ice-packs were used to cover the loin area of the carcasses in the 2°C chiller. Cold and warm ice-

packs were attached to the respective carcass loin area for 2 hours and then removed. At 3 and 24 hours post mortem, pH and temperature was recorded on both the right and left sides and a corresponding core for glucidic analysis was also collected. Chiller temperatures were maintained at 2°C and -2°C for 24 hours with air speeds at 0.5 m/sec, at which time both chiller temperatures were maintained at 2°C. Following 48 h of chilling, the *longissimus thoracis* (LT) muscles were removed from each carcass side, divided into anterior and posterior roasts, randomized to minimize location effect (Janz *et al.*, 2006) and assigned to 3 or 14 days ageing treatments (AGEING).

3.2.2 Meat Quality Measurements

At 3 days or following ageing (14 days), three 2.5 cm thick steaks were taken from each *longissimus thoracis* muscle.

3.2.2.1 Colour Analysis Measurements

The first steak was used for colour analysis following the same procedure described in section 2.2.2 of this thesis. Lightness (L^*), green-red (a^*) and blue–yellow (b^*) coordinates of the meat sample were obtained with a Minolta Chromameter CR-400 (Konica-Minolta, Osaka, Japan) using the colour system established by the Commission Internationale de L'Eclairage (CIE, 1976).

3.2.2.2 Warner Bratzler Shear Force Measurements and cook time

Warner–Bratzler Shear Force (WBSF) was calculated using the AMSA (AMSA 1995) method following the same procedure described in section 2.2.4 of this thesis. WBSF was measured using a material testing machine (AMETEK, Inc. Lloyd Instrument LRX plus, Digital Metrology Measurements, Kitchener, ON) fitted with a Warner–Bratzler type shear blade. Cook time (Chapter 2, section 2.2.3 of this thesis) was recorded every 3 minutes using a four-channel alarm timer (Traceable ®, Fisher Scientific. Edmonton, CA.), until steak internal temperature reached 71 °C. Cook time was calculated as minutes/100 g of fresh product.

3.2.2.3 pH Measurements

The third steak was used for pH measurements following the same procedure described in Section 2.2.5 of this thesis. A temperature-compensated pH meter was used (Fisher Scientific, Accumet Waterproof AP71 pH/mV/Temperature, Fisher Scientific, Toronto, ON) fitted with a glass probe electrode (Hanna Instruments, Fisher Scientific, Toronto, ON).

3.2.2.4 Water Holding Capacity Measurements

Following the same procedure described in Section 2.2.3 of this thesis, the third steak was used to determine the water holding capacity by drip loss as described by Honikel (1998) and cooking loss was assess as outlined by the American Meat Science Association methods (AMSA, 1995).

3.2.3 Carcass meat quality

3.2.3.1 Carcass pH, Temperature recording and glucidic sample collection

At 48 hours, carcasses were ribbed at the Canadian grade site (between the 12th and 13th ribs) and allowed a 20-minute "bloom" period. Right and left carcass sides were graded according to Canadian grade standards (Agriculture Canada. 1992). Following grading, final 48 hour pH and temperature was recorded. The portion of the LL with cores was removed from carcasses and a final 48 h glucidic sample collected.

3.2.3.2 Sarcomere length measurements

A sample of the right and left LT was taken to the laboratory and a 2 g sample removed, avoiding connective tissue and large deposits of fat. The 2 g sample was hand-minced with a surgical scalpel and homogenized in 20 mL of a 0.02M EGTA/0.25M sucrose solution (Polytron Homogenizer PT3100 and a 2 cm generator [Brinkmann Instruments Inc., Mississauga ON]). Three sarcomere lengths per image and ten images per meat sample were captured and measured (Axioscope, [Zeiss, West Germany] equipped with a Sony DXC 930 Color Video Camera, [Sony Corporation, Japan] and Image Pro-Plus software V4.0, [Mediacybernetics, Silver Spring, MD]).

3.2.3.3 Fibre typing and counting

A 1.0 cm^3 muscle sample was cut from the same steak, mounted on cork, perpendicular to the muscle grain with optimum cutting

temperature (OCT) compound and flash frozen in liquid nitrogen. The frozen block was then sectioned (11 μm thickness) using a Thermo Shandon Cryotome (Model 77210164 GB [Thermo Shandon Inc., Pittsburgh PA]) and slides double bagged and placed in a freezer at -35°C degrees overnight. The following day slides were removed from frozen storage and allowed to air dry approximately 2 hours prior to staining. Staining was performed according to Solomon and Dunn (1988) to differentiate between red (slow oxidative), white (fast glycolytic) and intermediate fibre (fast oxidative glycolytic) types using the basis of potential oxidative capacity. Images were captured and measurement analysis was performed as described above. For fibre typing and counting analyses; fibres of four bundles were typed and fibres of three bundles were measured per meat sample. Muscle fibre type results were expressed in percentage of total fibres and fibre measurements were expressed in μm^2.

3.2.3.4 Proximate analyses

At 15 days a steak from the anterior and posterior portion of both the left and right LT was ground (Robot Coupe Blixir BX3 [Robot Coupe USA Inc., Ridgeland MS]) and a 20 g subsample of the grind frozen at -20°C until analysis. Prior to analysis, frozen samples were removed from the freezer and thawed overnight in a cooler at 4°C. The following day samples were analyzed for moisture and fat using CEM rapid analyzer systems (Smart Turbo Moisture Analyzer Model 907990, and Smart Trac Fat Analyzer Model 907955 [CEM Corporation, Matthews, NC]) these machines utilize the AOAC Official Method 2008.06 for fat analysis and

the AOAC Official Methods 985.14 for moisture analysis. Results are expressed as a percentage of weight.

3.2.3.5 Glucidic Analysis

Glucidic metabolites were extracted from frozen muscle samples (Dalrymple and Hamm 1973) by pulverizing the sample with a mortar and pestle in liquid nitrogen. One g of the crushed sample was weighed into 5 ml of 0.6 N perchloric acid. The sample was homogenized for 30 seconds using a Polytron Homogenizer PT1200 with a 12 mm generator [Brinkmann Instruments Inc., Mississauga, ON]) and a 0.20 ml aliquot removed. Three (3M) potassium carbonate and amyloglucosidase (0.1 g) were added to the aliquot and the mixture placed in a 40°C water bath for 45 minutes for extraction of glycogen. Following extraction, the glycogen samples were centrifuged (Beckmann Floor Model J2-MC and JA-14 rotor [Beckmann Instruments Ltd., Mississauga ON]) and the supernatant neutralized with 3M potassium carbonate. Neutralized supernatant was analyzed for glycogen determinations using a glucose analyzer (YSI 2300 StatPlus, YSI Incorporated, Dayton OH). The remainder of the crushed homogenate was centrifuged and neutralized with perchloric acid, to be used for free glucose and lactate analysis. Glucidic metabolites were calculated by multiplying the weight of sample (g) by the corresponding dilution factor (Dalrymple and Hamm, 1973) and reported as μmol/g. The total sum of free glucose, glycogen, and half of lactate measurements [(lactate/2) + glucose + glycogen] was used to estimate the total amount of energy available at slaughter (glucidic potential).

3.2.4 Statistical Analyses

Data were analyzed as a split-split-plot using the MIXED procedure in the Statistical Analysis System (SAS) software (Version 9.2, Statistical Analysis Systems, Cary, NC, USA). Analysis of variance was conducted using TEMP, ES and AGEING as fixed effects. Carcasses were blocked by replicate and block was included as a random source of variation. Treatment effects were considered significant at $P \leq 0.05$ and where treatment was significant least square mean differences were used to determine differences between means with significance at $P \leq 0.05$. Degrees of freedom were corrected using the Satterthwaite adjustment. Repeated measurements analysis was perform on data that was collected over time and Chi Square analyses was utilized to determine the probability distribution of the glucidic analyses data. Correlations analyses were performed using PROC CORR to obtain Pearson correlations among the meat quality data and to determine linear dependence among variables. PROC CORR was also performed to identify a possible correlation of lactate and pH levels.

3.3 RESULTS

3.3.1 Early Post-mortem Muscle Biochemistry

3.3.1.1 Temperature Decline

Chiller temperature and its interaction with time had an effect on carcass temperature decline ($P < 0.0001$). Those carcasses that were railed into the -2°C chiller had lower LL temperatures at 1 ($P = 0.0483$), 3 ($P <$

0.0001), and 24 (P < 0.0001) h post mortem than carcasses railed into the 2°C chiller, with no difference in intramuscular LL temperature between treatments at 48 h (P = 0.1548) (Table 3-1).

1.1.1.1 pH Decline

Electrical stimulation and its interaction with time had an effect (P < 0.0001) on mean intramuscular pH decline as carcasses that received electrical stimulation had lower LL pH values at 1 (P < 0.0001), 3 (P < 0.0001), and 24 (P = 0.0003) h post mortem than those of non-electrically stimulated carcasses, while at 48 h there was no difference in intramuscular mean LL pH value between treatments (P = 0.09) (Table 3-2).

3.3.1.2 Glucidic Analyses

3.3.1.2.1 Glucose

Chiller temperature and its interaction with time had a trend effect on muscle glucose content (P = 0.0681). Carcasses railed into the 2°C chiller tended to have higher glucose content than those railed into the -2°C chiller (Table 3-3), whereas electrically stimulated carcasses had higher (P = 0.0015) muscle glucose content than those of non-electrically stimulated LL at 1 (P < 0.0001), 3 (P < 0.0001), 24 (P < 0.0001) and 48 (P < 0.0001) hours post mortem (Table 3-3).

3.3.1.2.2 Lactate

Electrical stimulation and its interaction with time had an effect (P < 0.0001) on lactate content as carcasses that received electrical stimulation had a higher mean intramuscular lactate content at 1 (P < 0.0001), 3 (P <

0.0001), 24 (P = 0.0010) and 48 (P = 0.0042) h post mortem than those of non-electrically stimulated carcasses (Table 3-4).

3.3.1.2.3 Glycogen

Chiller temperatures and its interaction with time had an effect on muscle glycogen content (P = 0.0476) only at 1 hour post mortem (P = 0.0252), as carcasses railed into the 2°C chiller had higher glycogen content than those railed into the -2°C chiller (Table 3-5). Also, electrically stimulated carcasses had lower (P < 0.0001) muscle glycogen levels than those non-electrically stimulated at 1 (P = 0.0434) and 3 (P = 0.0091) but there were no differences at 24 (P = 0.3189) and 48 (P = 0.8610) h post mortem (Table 3-5).

3.3.1.2.4 Glucidic Potential

Glucidic potential was affected by electrical stimulation only at 24 hours post mortem (P= 0.0201), as carcasses that were electrically stimulated had a higher glucidic potential than those from non-electrically stimulated carcasses (Table 3-6).

3.3.2 Carcass and Meat Quality

3.3.2.1 Live and Carcass Weights

All carcasses graded AA (left side). Live weight ranged from 438 to 595 kg and carcass weights ranged from 251 to 335 kg.

3.3.2.2 Meat Colour

Chiller temperature had an effect on meat colour, with carcasses that were railed into the 2°C chiller producing steaks with higher L* values (P = 0.03) and b* (P = 0.02) values than those of -2°C. Ageing had a significant effect on colour, with 14 days aged roasts producing steaks with higher L* (P < 0.0001), a* (P < 0.0001) and b* (P = 0.04) values than those that were not aged (Table 3-7). Electrical stimulation had also an impact on colour, as steaks from electrically stimulated carcasses had higher values of a* (P = 0.0006) and b* (P = 0.0009) than those steaks from non-electrically stimulated carcasses (Table 3-8).

3.3.2.3 Ultimate pH

The interaction of chiller temperatures and electrical stimulation had an effect on muscle pH (P = 0.03). Those carcass sides that were railed into the 2°C chiller and received electrical stimulation produced steaks with higher pH (P = 0.02) than those of non-electrically stimulated carcass sides (Table 3-10). Ageing had a significant effect on pH, with roasts that were aged for 14 days producing steaks with higher pH (P < 0.0001) than those from non-aged roasts (Table 3-9).

3.3.2.4 Drip Loss

Ageing had a significant effect on drip loss, as roasts that were aged for 14 days produced steaks with lower mean drip loss (P <0.0001) than that of steaks from non-aged roasts (Table 3-9). There was a trend (P = 0.08) toward significance for the interaction between electrical stimulation and ageing, where steaks from carcasses that received electrical

stimulation at 14 days (P = 0.06) had a lower mean drip loss than those that did not receive electrical stimulation (Table 3-10).

3.3.2.5 Cook Loss

Ageing had a significant effect on cook loss (P = 0.0123), as roasts that were aged for 14 days produced steaks with lower cook loss (P = 0.0123) than those from non-aged roasts (Table 3-9).

3.3.2.6 Warner-Bratzler Shear Force

A significant interaction between electrical stimulation and ageing indicated that steaks from electrically stimulated carcasses at 3 days had lower values of Warner-Bratzler shear force (P = 0.0079) than those of non-electrically stimulated carcasses; however this difference did not persist at day 14 (Table 3-10).

3.3.2.7 Sarcomere Length

There were no effects of chiller temperatures, electrical stimulation or their interaction on sarcomere length.

3.3.2.8 Fibre typing and counting

There were no effects of chiller temperatures, electrical stimulation or their interaction on fibre type or percentage of fibre type. There was a trend, however, for the interaction between electrical stimulation and chiller temperature (P = 0.0617) to influence white fibre percentage in muscle, with non-electrically stimulated carcasses railed into the 2°C chiller tending to have an increased percentage of white fibre in muscle.

3.3.2.9 Proximate Analyses (moisture and fat content)

Muscle location (anterior and posterior) had an effect on fat content (P = 0.0048) with the anterior part of the muscle having higher fat content than those from the posterior part. Chiller temperature and electrical stimulation did not have an impact on moisture content.

3.3.3 Correlation among meat quality traits of beef *m. longissimus thoracis* (rib eye)

Significant correlations were found and are reported in Table 3-11. L* at 3 days was correlated (r = 0.71) to L* at 14 days. L* at 14 days was correlated (r = 0.46) to b* at 14 days and a* at 3 days was correlated (r = 0.73) to b* at 3 days. Also, a* at 14 days was correlated (r = 0.73) to b* at 14 days. Warner-Bratzler shear force (WBSF) at 3 days was correlated (r = 0.47) to WBSF at 14 days. Moisture at 3 days was correlated to moisture at 14 days (r = 0.59), Fat at 3 days (r = -0.84) and fat at 14 days (r = -0.50). Moisture at 14 days was correlated to fat at 3 days (r = -0.49) and fat at 14 days (r= -0.86). Fat at 3 days was correlated to fat at 14 days (r = 0.51).

3.3.3.1Correlation of Lactate and pH values

Lactate was highly correlated to pH (r = -0.94).

3.4 DISCUSSION

Electrical stimulation, early post mortem chill temperatures and post mortem ageing have been used extensively to control and manipulate beef tenderness and quality (Calkins *et al*., 1980; Aalhus *et al.,* 2001), and relationships between early post mortem pH and temperature are well known (Ducastaing *et al*., 1985; Aalhus *et al.,* 2001; Hwang *et al*., 2001). Electrical stimulation has been applied to beef carcasses both early (within 5 minutes) and late (within 60 minutes) post mortem, with low voltages used early and high voltages used late post mortem due to the decline in the functionality of the nervous system with time after death (Swatland, 1982). Both low and high voltage electrical stimulation are used routinely in beef carcass processing facilities to increase tenderness and brighten muscle colour (Calkins *et al*., 1980; Aalhus *et al.,* 2001). The reduction of early post mortem chill temperatures has been prompted by the desire to increase carcass throughput in order to reduce the amount of cost per carcass processed. How far early post mortem chill temperatures can be decreased is limited by the potential for cold shortening and toughening of the external muscles (Aalhus *et al.,* 2001). Also, beef ageing is an effective tenderizer in normal, non-cold shortened beef (Calkins *et al*., 1988; Monsón *et al*., 2004; Gruber *et al*. 2006). Despite the effectiveness of these methods being well-known, the best combination of these methods has not been elucidated and eludes both researchers and processors.

In the present study, the early post mortem chill temperature treatments resulted in significant differences in *longissimus lumborum* muscle temperature decline, and high voltage electrical stimulation reduced pH values during the first 24 h, indicating that experimental

treatments were effective. Several studies (Eikelenboom *et al.*, 1985; Koh *et al.*, 1987; Aalhus *et al.*, 1992; Geesink *et al.*, 2001; Janz *et al.*, 2001) have showed that electrical stimulation improves meat quality and helps to avoid cold shortening. This study showed that electrical stimulation significantly increased meat redness and tenderness at 3 days, but that this improvement declined with time *post mortem.* These findings agree with those of Hwang *et al.* (2001) who found that electrical stimulation accelerated ageing mechanisms, and with those of Li *et al.* (2011) who indicated that electrical stimulation improved meat colour, and that the differences in meat quality decreased over time. In this study, electrical stimulation accelerated rigor mortis in the LL as indicated by the early post mortem LL muscle pH, which according to Bendall (1973) occurs at pH 5.9. Assuming that Bendall (1973) was correct, then in the present study rigor mortis occurred at about 2.5 h post mortem for the electrically-stimulated carcasses while muscle temperature was still high at approximately 30°C. This combination of low pH and high temperature early post mortem may have promoted increased proteolysis of the muscle as reported by Young *et al.* (1999) and improved colour as proteolysis increases colour reflectance either through structural alteration of the myofibrillar proteins (Sleper *et al.*, 1983) or increased water exudation (Scopes, 1964; Scopes, 1970). The differences in colour and tenderness may have decreased with ageing time due to post *mortem* proteolysis of the cytoskeleton proteins that connect the myofilaments with the sarcolemma (Huff-Lonergan *et al.*, 2005), specifically the degradation of the proteins nebulin, titin, filamin, desmin and troponin-T by the calpain system (μ-calpain primarily) (Huff-Lonergan *et al.*, 1996).

Increased proteolysis has been associated which will increase colour reflectance and tenderization of the meat (Koohmaraie *et al.*, 1987).

In terms of electrical stimulation and its interaction with chiller temperatures, statistical differences were found in the L* and b* values of the LT muscles due to early post mortem chill temperature, with carcasses exposed to warm temperatures (+2°C) having LT that was lighter and more yellow than that exposed to cold temperatures (-2°C). These findings agreed with those of Bruce and Ball (1990), which showed that increased lightness of lean was related to warm muscle temperatures early post mortem. Differences in the glycolytic rate as demonstrated by glucose, lactate and glycogen levels over time carcasses with a faster glycolysis may also have early proteolysis of myofibrillar proteins (O'Halloran *et al.*, 1995). Although differences in meat colour were statistically different, these differences in colour may not be detectable by the human eye.

3.5 CONCLUSION

The results of this study indicate that electrical stimulation may be used on carcasses to accelerate the rigor mortis and when combined with increased chill rate, carcasses can be processed quickly without the risk of cold shortening, or of reducing water holding capacity. Electrical stimulation also accelerated ageing mechanisms that can potentially reduce the amount of time required to deliver a tender and attractive product to consumers, thus saving valuable energy resources such as electricity and handling costs due to the reduced storage time.

3.6 TABLES

Table 3-1 Carcass (*m. longissimus lumborum*) temperature decline affected by chiller temperature and its interaction with time *post mortem*

	Chiller Temperature			
Carcass temperature Over time	- 2°C	+2°C		
n	20	20	Pr > F[1]	SEM[2]
1 hour	39.98 °C	39.78 °C	0.0483	0.0954
3 hours	20.62 °C	24.72 °C	<.0001	0.5650
24 hours	0.56 °C	2.09 °C	<.0001	0.1865
48 hours	2.67 °C	2.88 °C	0.1548	0.1445

[1] Probability of the calculated F value with significance at $P \leq 0.05$

[2] SEM - Standard error of the mean

Table 3-2 Carcass (*m. longissimus lumborum*) pH decay affected by electrical stimulation and its interaction with time *post mortem*

	Electrical Stimulation (ES)			
carcass pH over time	ES[1]	Non-ES[2]		
n	20	20	Pr > F[3]	SEM[4]
1 hour	6.27	6.74	<.0001	0.03
3 hours	5.52	6.20	<.0001	0.05
24 hours	5.56	5.65	0.0003	0.02
48 hours	5.47	5.49	0.0904	0.01

[1]ES - Electrical Stimulation

[2] Non-ES – Non-Electrically Stimulated

[3] Probability of the calculated F value with significance at $P \leq 0.05$

[4] SEM - Standard error of the mean

Table 3-3 Carcass glucose (*m. longissimus lumborum*) profile affected by electrical stimulation and chiller temperature over time *post mortem*

	TREATMENTS							
	Electrical stimulation (ES)				Chiller Temperature			
Glucose µmol/g	ES[1]	Non-ES[2]	Pr > F[3]	SEM[4]	-2°C	+2°C	Pr > F[3]	SEM[4]
n	20	20			20	20		
1 hour	1.67	0.37	<0.0001	0.09	1.04	0.99	0.6290	0.09
3 hours	4.02	1.63	<0.0001	0.27	2.59	3.07	0.0884	0.27
24 hours	5.57	4.39	<0.0001	0.18	4.84	5.12	0.1262	0.18
48 hours	5.85	4.70	<0.0001	0.16	5.11	5.44	0.0503	0.16

[1]ES - Electrical Stimulation

[2] Non-ES – Non-Electrically Stimulated

[3] Probability of the calculated F value with significance at $P \leq 0.05$

[4] SEM - Standard error of the mean

Table 3-4 Carcass lactate (*m. longissimus lumborum*) profile affected by electrical stimulation over time *post mortem*

	Electrical stimulation (ES)			
Lactate μmol/g	ES[1]	Non-ES[2]		
n	20	20	Pr > F[3]	SEM[4]
1 hour	45.44	21.09	<0.0001	1.64
3 hours	92.97	58.12	<0.0001	4.94
24 hours	111.83	101.57	0.0010	2.86
48 hours	112.52	105.73	0.0042	2.22

[1]ES - Electrical Stimulation

[2] Non-ES – Non-Electrically Stimulated

[3] Probability of the calculated F value with significance at $P \leq 0.05$

[4] SEM - Standard error of the mean

Table 3-5 Carcass glycogen (*m. longissimus lumborum*) profile affected by electrical stimulation and chiller temperature over time *post mortem*

	TREATMENTS							
	Electrical stimulation (ES)				Chiller Temperature			
Glycogen µmol/g	ES[1]	Non-ES[2]	Pr > F[3]	SEM[4]	-2°C	+2°C	Pr > F[3]	SEM[4]
n	20	20			20	20		
1 hour	68.27	82.51	0.0434	6.80	67.45	83.34	0.0252	6.80
3 hours	44.86	57.60	0.0091	4.62	49.52	52.94	0.4635	4.62
24 hours	31.97	28.73	0.3189	3.20	27.22	33.48	0.0586	3.20
48 hours	30.20	30.77	0.8610	3.26	28.25	32.72	0.1791	3.26

[1]ES - Electrical Stimulation

[2] Non-ES – Non-Electrically Stimulated

[3] Probability of the calculated F value with significance at $P \leq 0.05$

[4] SEM - Standard error of the mean

Table 3-6 Carcass glucidic potential (*m. longissimus lumborum*) affected by electrical stimulation over time *post mortem*

	Electrical stimulation (ES)			
Glucidic Potential μmol/g	ES[1]	Non-ES[2]		
n	20	20	Pr > F[3]	SEM[4]
1 hour	92.67	93.43	0.9158	7.16
3 hours	95.37	88.30	0.1930	5.33
24 hours	93.46	83.91	0.0201	3.92
48 hours	92.32	88.35	0.3276	4.00

[1]ES - Electrical Stimulation

[2] Non-ES – Non-Electrically Stimulated

[3] Probability of the calculated F value with significance at $P \leq 0.05$

[4] SEM - Standard error of the mean

Table 3-7 Chiller temperature effect on meat quality

Analysis	Chiller Temperature		Pr > F[1]	SEM[2]
	-2°C	+2°C		
n	40	40		
L*	36.77	38.24	0.0353	0.46
a*	20.61	20.87	0.5726	0.41
b*	3.69	4.36	0.0285	0.21
Drip Loss (%)	1.11	1.10	0.8150	0.04
Cook Loss (%)	18.86	19.23	0.7341	0.67
Cook Time (minutes)	2.85	2.82	0.8318	0.11

[1] Probability of the calculated F value with significance at $P \leq 0.05$

[2] SEM - Standard error of the mean

Table 3-8 Electrical stimulation effect on meat quality

Analysis	Electrical Stimulation (ES)		Pr > F[3]	SEM[4]
	ES[1]	Non-ES[2]		
n	40	40		
L*	38.13	36.88	0.1156	0.35
a*	21.22	20.26	0.0006	0.37
b*	4.55	3.50	0.0009	0.21
Drip Loss (%)	1.08	1.13	0.3669	0.04
Cook Loss (%)	18.71	19.38	0.1231	0.51
Cook Time (minutes)	2.93	2.74	0.3933	0.13

[1]ES - Electrical Stimulation

[2] Non-ES – Non-Electrically Stimulated

[3] Probability of the calculated F value with significance at $P \leq 0.05$

[4] SEM - Standard error of the mean

Table 3-9 Ageing effect on meat quality

Analysis	Ageing Period		Pr > F[1]	SEM[2]
	3 days	14 days		
n	40	40		
L*	36.50	38.51	<0.0001	.35
a*	20.17	21.31	<0.0001	.37
b*	3.72	4.33	0.0487	.21
Drip Loss (%)	1.24	.98	<0.0001	.04
Cook Loss (%)	19.55	18.54	0.0123	.51
Cook Time (minutes)	2.96	2.71	0.0227	.1087

[1] Probability of the calculated F value with significance at $P \leq 0.05$

[2] SEM - Standard error of the mean

Table 3-10 Electrical Stimulation, chiller temperature and ageing treatment interactions effects on beef quality

	INTERACTIONS									
	-2°C	-2°C	+2°C	+2°C	3days	3days	14days	14days		
Analysis	ES[1]	non-ES[2]	ES[1]	non-ES[2]	ES[1]	non-ES[2]	ES[1]	non-ES[2]	Pr > F[3]	SEM[4]
n	20	20	20	20	20	20	20	20		
a*	-	-	-	-	20.9 [x]	19.4 [y]	21.5 [x]	21.07 [x]	0.0680	.41
pH	5.52 [ab]	5.53 [ab]	5.54 [a]	5.51 [b]	-	-	-	-	0.0350	.01
Drip Loss (%)	-	-	-	-	1.26 [x]	1.21 [x]	.90 [y]	1.05 [y]	.0813	.05
WBSF[5] (Newton)	-	-	-	-	34.6 [a]	44.77 [b]	25.32 [c]	28.31 [c]	0.0079	1.5
WBSF[5] (Newton)	28.8 [x]	37.8 [y]	31.0 [z]	35.2 [y]	-	-	-	-	.0904	1.75

[1]ES - Electrical Stimulation

[2] Non-ES – Non-Electrically Stimulated

[3] Probability of the calculated F value with significance at $P \leq 0.05$

[4] SEM - Standard error of the mean

[5] WBSF - Warner-Bratzler Shear Force

[a,b,c] Means with different superscripts within a row are significantly different at $P < 0.05$ according to least square mean differences tests.

[x,y,z] Means with different superscripts within a row are significantly different at $P < 0.1$ according to least square mean differences tests.

Table 3-11 Pearson Correlation coefficients among selected meat quality traits of beef *m. longissimus thoracis* (Rib Eye) from 3 and 14 days ageing

Variable	L*-3 days	L*-14 days	a*-3 days	a*-14 days	b*-3 days	b*-14 days	WBSF[1] - 3 days	WBSF[1] - 14 days	Moisture-3 days	Moisture-14 days	Fat-3 days	Fat-14 days
n	40	40	40	40	40	40	40	40	40	40	40	40
L*-3 days	1.00	0.71**	0.12	-0.05	0.45	0.42	0.01	0.04	-0.11	0.03	0.20	0.13
L*-14 days		1.00	-0.03	-0.13	0.23	0.46*	-0.15	0.01	-0.08	0.01	0.11	0.12
a*-3 days			1.00	0.27	0.73**	0.11	-0.33	-0.17	-0.44	-0.26	0.37	0.15
a*-14 days				1.00	-0.15	0.73**	0.00	-0.10	-0.31	-0.44	0.12	0.42
b*-3 days					1.00	-0.13	-0.26	-0.10	-0.25	-0.17	0.32	0.15
b*-14 days						1.00	0.00	-0.01	-0.27	-0.26	0.10	0.32
WBSF[1] -3 days							1.00	0.47*	0.21	0.35	-0.28	-0.36
WBSF[1] -14 days								1.00	0.18	0.23	-0.21	-0.28
Moisture-3 days									1.00	0.59**	-0.84**	-0.50*
Moisture-14 days										1.00	-0.49*	-0.86**
Fat-3 days											1.00	0.51*
Fat-14 days												1.00

[1] WBSF - Warner-Bratzler Shear Force

* P= ≤0.0025

** P=<0.0001

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Chapter 4

4.0 Meat Quality Description of *M. Longissimus Thoracis* of the Canada A, AA, AAA & Prime Grades

4.1 INTRODUCTION

In a previous study (Chapter 2) examining differences in beef quality associated with Canada quality grade, the quality differences among Canada A, AA, AAA and Prime grades indicated that differences in beef quality were limited between the grades. This result was unexpected because the Canada quality grades are assigned based upon the amount of visible intramuscular fat, which is supposedly associated with eating quality (Simone *et al*., 1959). The insufficiency of replication in that study was denoted in a subsequent power analysis, which showed that the power of the analysis was 0.60, much less than the desired 0.80. Furthermore, the power analysis suggested doubling the quantity of samples to a total of 96 to provide a power level of 0.82. Although differences in some meat quality traits were identified between the Canada quality grades in the first study, having additional samples may allow us to elucidate representative differences in beef quality indicators between the high quality Canada grades. In a previous study in the United States on the influence of marbling on beef quality (McBee *et al*., 1967), eight different degrees of marbling with 40 carcasses per each degree of marbling were used. The McBee *et al.* (1967) study reported that

marbling had an effect on shear force values, with steaks with the highest marbling (marbling degree 8, United States Department of Agriculture [USDA] Prime) having the lowest mean shear force value and steaks with the lowest marbling (marbling degree 1, USDA Standard) having the highest mean shear force value. Moreover, a large amount of variation between the USDA beef grades was observed. The Canadian beef grading system is similar to that of the United States; therefore, in this experiment results similar to those of McBee et al. (1967) were expected. This study tested the hypothesis that both marbling and Warner-Bratzler shear force are different among the Canada grades, with toughness decreasing as marbling increases.

4.2 MATERIALS AND METHODS

4.2.1 Experimental Design

The experimental design was as described in Chapter 2 Section 2.2.1, with the exception that the anterior and posterior portions of the *m longissimus thoracis* (LT, rib eye) were randomized between the non-aged and aged 14 days treatments, and the packing date printed on the label of each meat box was recorded to assist with precise interpretation of results. Following each ageing treatment, three 2.5 cm thick steaks were sliced from the posterior of each LT muscle section; the first was used for objective colour measurements and proximate analyses, the second was used for cooking loss and Warner–Bratzler Shear Force (WBSF) and the third was used for pH and drip loss.

4.2.2 Colour Analysis Measurements

The first steak was used for colour analysis following the same procedure described in Section 2.2.2 of this thesis. Lightness (L^*), green-red (a^*) and blue–yellow (b^*) coordinates of the meat sample were measured and calculated with a Minolta Chromameter CR-400 (Konica-Minolta, Osaka, Japan) using the colour system established by the Commission Internationale de L'Eclairage (CIE, 1976). Colour of the lean was estimated with three measurements each in a different location on the face of the LT muscle that had been allowed to 'bloom' for at least 20 minutes at 4°C.

4.2.3 Cooking loss, cooking time and Warner-Bratzler shear force

The second steak was trimmed of subcutaneous fat, weighed and grilled on a pre-heated grill (General Electric 4 in 1 Grill / Griddle, China) set to a temperature of 176 °C. The internal temperature of each steak was monitored continuously using a thermometer with a metal probe (Tinytag View 2s, manufacturer, city) inserted into the geometric centre of the steak. Steaks were heated until the steak internal temperature reached 71 °C. Once an internal temperature of 71 °C was reached, the cooked steaks were cooled to less than 10 °C in an ice bath and then weighed the next day after cooling. Cooking loss was calculated by dividing the steak weight loss during cooking by the trimmed raw weight of the steak and reported as a percentage of the initial raw weight. Cooking time was recorded every 3 minutes until steak internal temperature reached 71 °C using a four-channel alarm timer (Traceable ®, Fisher Scientific, Edmonton, CA.), and cook time was calculated as minutes/100 g of fresh product. Cooked steaks were stored overnight at 4 °C, and the following day steaks were removed from refrigerated storage and allowed to

reach room temperature. Once the cooked steaks reached room temperature, 6 cores of 1.27 cm diameter and 2 cm long were removed from each steak parallel to the muscle fibers using a cork borer. Each core was sheared once across the middle, perpendicular to the fiber direction, using a materials testing machine (AMETEK, Inc. Lloyd Instrument LRX plus, Digital Metrology Measurements, Kitchener, ON) fitted with a Warner–Bratzler type shear blade travelling at 225 mm/min. Shear force was expressed in Newtons (N) and values were averaged to obtain a mean value for each steak.

4.2.4 pH Measurements

The third steak was used for pH measurements following the same procedure described in Section 2.2.5 of this thesis. A temperature-compensated pH meter was used (Fisher Scientific, Accumet Waterproof AP71 pH/mV/Temperature, Fisher Scientific, Toronto, ON) fitted with a glass probe electrode (Hanna Instruments, Fisher Scientific, Toronto, ON). Three measurements were taken at three different areas of the muscle and the pH probe was standardized using pH 4 and 7 standards (Fisher Scientific, Toronto, ON) at room temperature (22 °C). The mean pH value of the three measurements from each steak was used for statistical analyses.

4.2.5 Water Holding Capacity Measurements

Following the same procedure described in Section 2.2.3 of this thesis, the third steak was used to estimate the water holding capacity of the muscle by drip loss as described by Honikel (1998) and as recommended by the American Meat Science Association methods (AMSA, 1995).

4.2.6 Proximate Analyses

To determine the chemical composition of our beef samples, approximately 100 g of minced sample were weighed and placed into an aluminium tray and lyophilized for four days using a freeze dryer (Virtis freeze dryer ultra El-85, SP Scientific, Warminster, PA., USA.). Upon the completion of lyophilisation, the trays were removed from the freeze dryer and final weights recorded for moisture loss calculation. Crude fat analysis was determined using samples that were lyophilized and then ground into a fine powder using a blender fitted with a 750 mL stainless steel container (Two-Speed Food Blender, Model 7011G; Waring Commercial. Torrington, CT., USA). Duplicate samples of this dry meat powder of approximately 2 g each were placed in cellulose thimbles and analyzed for crude fat content (Method 960.39; Association of Official Analytical Chemists 1995) by petroleum ether extraction (Foss Soxtec System Model 2050; Foss Analytical AB, Hoganas, Sweden). A single blank/control sample was included in each analysis to correct for machine contamination and duplicates were averaged to supply a mean value for statistical analysis. Nitrogen content was determined using triplicates of 100 mg of grinded dry meat sample (Method 992.15; Association of Official Analytical Chemists 1997) (Nitrogen/Protein Determinator CNS2000, Leco Corp., St., Joseph, MI., USA.) and triplicates were averaged and means used for statistical analysis. Standardization and calibration of the Leco machine was performed with three replicates of caffeine and ethylenediaminetetraacetic acid (EDTA) samples prior to meat sample analyses.

4.2.7 Statistical Analyses

Data were organized as a randomized split plot complete block design and analyzed using the MIXED procedure in the Statistical Analysis System (SAS) software (Version 9.2, Statistical Analysis Systems, Cary, NC, USA). *Longissimus thoracis* muscles were used as the whole plot experimental unit and roasts subjected to ageing treatments were used as the sub-plot experimental unit. The data "*post mortem* time", which was the number of days post mortem collected from the beef boxes, was used as a covariate in the model. Correlations analyses were performed using PROC CORR to obtain Pearson correlations among the meat quality data to calculate possible linear dependence among variables. PROC REG was used to perform multiple regression and relate meat quality measurements to Warner-Bratzler shear force values, and only non-correlated variables as determined by PROC CORR were included in the model. For multiple regression the stepwise model fit function was used and the level of significance for entry into the model was $P < 0.05$.

4.3 RESULTS

4.3.1 Colour

There were no differences among Canada beef grades for L* (lightness) values (Table 4-1). However, L* values were affected by ageing; 14 days aged steaks had higher L* values (P= 0.0018) than 3 days aged steaks (Table 4-3). Values of a* (redness) were affected by an interaction of Canada beef grade and ageing (P= 0.0259), where Canada AA, AAA and Prime at 14 days were more reddish than those from the same grade at 3

days. At 14 days Canada Prime had the highest a* and b* values from all Canada grades, followed by Canada AAA (Table 4-2). Values of b* (yellowness) were also affected by the interaction of Canada grade and ageing. At 3 and 14 days Canada Prime was the most yellow from all grades, whereas at 3 days no difference in yellowness was found among Canada A and Canada AA, although at 14 days Canada AA was more yellow than Canada A (Table 4-3).

4.3.2 Ultimate pH

Differences in meat pH were found in ageing treatments (P = 0.0328) was detected for 14 days aged beef to have a greater mean pH value than that of 3 days aged beef (Table 4-3). However, this difference consisted of only 0.02 pH units.

4.3.3 Drip Loss

Differences between the Canada grades in terms of drip loss were detected (P = 0.0011), with Canada Prime having a lower mean drip loss than steaks from the Canada AAA, Canada AA and Canada A grades. Also, Canada A and Canada AA steaks had a higher mean drip loss than steaks from Canada AAA and Canada Prime (Table 4-1). A trend (P = 0.0781) was found for 14 days aged steaks having less drip loss than those of 3 days aged beef.

4.3.4 Cook Loss

Ageing treatments affected cook loss, with 14 days aged steaks having a higher mean cook loss than those from 3 days aged beef (Table 4-3).

4.3.5 Cook Time

Differences in cook time were limited to trends only (P = 0.0679). A trend was exhibited as result of an interaction effect between Canada grades and ageing with the non-aged rib eye steaks from the Canada Prime grade tending to take longer to be cooked than those of Canada A (Table 4-2).

4.3.6 Warner-Bratzler Shear Force

Differences between the Canada grades for Warner-Bratzler shear force were limited to trends (P = 0.0731) (Table 4-1); however, differences between ageing treatments (P < 0.0001) were found. At 14 days, steaks had lower Warner-Bratzler shear force values than those from 3 days aged beef (Table 4-3).

4.3.7 Proximate analyses

4.3.7.1 Crude Fat Content

Differences were found in crude fat content among the Canada grades (P < 0.0001), with Canada Prime having the highest fat content followed by Canada AAA. There was no statistical difference between steaks from the Canada A and Canada AA grades (Table 4-1).

4.3.7.2 Protein Content

Differences were found in protein content among the Canada grades (P < 0.0001), with Canada Prime having the lowest protein content. Canada AA had higher protein

content than Canada AAA but not higher than Canada A grade (Table 4-1). Ageing also affected protein (P = 0.0139) content as 14 days aged beef had a higher mean protein content than that of 3 days aged beef.

4.3.7.3 Moisture Content

Differences were found in moisture content among the Canada grades (P < 0.0001), with Canada Prime having the lowest moisture content followed by Canada AAA. No difference was found between Canada A and Canada AA (Table 4-1). A trend (P = 0.0601) was found for 3 days aged beef to have higher mean moisture content than that of 14 days aged beef.

4.3.8 Correlation among meat quality traits of beef *m. longissimus thoracis* (Rib Eye)

For the data collected at 3 days, significant correlations were found and are reported in Table 4-4. L* was correlated to a* (r = -0.30), b* (r = 0.37), ultimate pH (r = -0.42), and cook loss (r = -0.28). Colour a* value was correlated b* (r = 0.66), Warner-Bratzler shear force (r = -0.45), crude fat (r = 0.67), moisture (r = -0.61) and protein (r = -0.53). b* was correlated to drip loss (r = -0.28), Warner-Bratzler shear force (r = -0.34), crude fat (r = 0.51), moisture (r = -0.47) and protein (r = -0.44). Drip loss was correlated to crude fat (r = -0.39) and moisture (r = 0.36). Cook loss was correlated to Warner-Bratzler shear force (r = 0.44), crude fat (-0.31) and moisture (r = 0.31). Warner-Bratzler shear force was correlated to crude fat (r = -0.28) and protein (r = 330.34). Cook time was correlated

to crude fat (r = 0.31) and moisture (r = -0.34). Crude fat was correlated to moisture (r = -0.98) and protein (r = -0.59). Moisture was correlated to protein (r = 0.51).

At 14 days significant correlations were found and are reported in Table 4-5. L* was correlated to a* (r = -0.29), b* (r = 0.40) and pH (r = -0.50). a* was correlated to b* (r = 0.68), drip loss (r = -0.35), cook loss (r = -0.36), crude fat (r = 0.63), moisture (r = -0.58) and protein (r = -0.49). b* was correlated to crude fat (r = 0.60), moisture (r = -0.60) and protein (r = -0.43). Drip loss was correlated to crude fat (r = -0.55), moisture (r = 0.52) and protein (r = 0.37). Cook Loss was correlated to Warner-Bratzler shear force (r = 0.54). No correlations were found for cook time. Crude fat was correlated to moisture (r = -0.97) and protein (r = -0.68). Moisture was correlated to protein (r = 0.59).

Correlation of data from 3 and 14 days beef are reported in table 4-6 where L*-3 days was correlated to L*-14 days (r = - 0.82) and pH-14 days (r = - 0.51). Colour value a*-3 days was correlated to a*-14days (r = 0.68) b*-14 days (r = 0.44), fat-14 days (r = 0.63), moisture-14 days (r = - 0.57) and protein-14 days (r = - 0.52). Colour value b*-3 days was correlated to b*-14 days (r = 0.58), fat-14 days (r = 0.49) and moisture-14 days (r = - 0.45). Intramuscular ultimate pH-3 days was correlated to ultimate pH-14 days (r = 0.49). Fat-3 days was correlated to a*14 days (r = 0.62), b*-14 days (r = 0.61), drip loss-14 days (r = -0.57) fat-14 days (r = 0.95), moisture-14 days (r = - 0.94) and protein-14 days (r = - 0.64). Moisture-3 days was correlated to a*14 days (r = - 0.58), b*-14 days (r = - 0.60), drip loss-14 days (r = 0.58) fat-14 days (r = - 0.93), moisture-14 days (r = 0.95) and protein-14 days (r = 0.56). Protein-3 days was correlated to fat-14 days (r = - 0.56), moisture-14 days (r = 0.46) and protein-14 days (r = 0.59).

4.3.9 Multiple regression

Table 4-7 shows results for multiple regression models. The multiple regression statistical analysis of Warner-Bratzler shear force at 3 days (R^2 = 0.2092) used the colour value a* only, while Warner-Bratzler shear force at 14 days (R^2 = 0.2990) used cooking loss as the only variable to predict shear force. Warner-Bratzler shear force prediction at any given time (3 or 14 days) included b* and cooking loss produced a model (R^2 = 0.3016) to predict shear force.

Regression equations for Warner-Bratzler shear force were:

Regression equation for Warner-Bratzler shear force values at 3 days=

83.90974 -1.88002*(a*)

Regression equation for Warner-Bratzler shear force values at 14 days=

9.35171-1.10230*(cooking loss)

Regression equation for Warner-Bratzler shear force using b* and cooking loss=

38.01767 -2.71900*(b*)+0.96723*(cooking loss)

4.4 DISCUSSION

Unlike in the first experiment, Canada grade did not have an effect on *L** values for steak lean regardless of the amount of intramuscular fat related to each grade, and this may be related to the extra attention at measurement to avoid marbling when the

measurements were taken. Steaks from *m. longissimus thoracis* (LT) of Canada Prime grade had the highest mean a* value at 3 and 14 days of ageing and the highest mean b* values at 14 days of ageing, and this may have been due to increased subcutaneous fat associated with the Canada Prime carcasses. Previous research has shown that subcutaneous fat contributes to warm muscle temperatures early *post mortem*, and increased subcutaneous fat can be associated with carcasses that have high levels of marbling (Moon *et al*., 2006). A high early post mortem temperature can also denature muscle proteins, which could increase colour reflectance either through the structural alteration of the myofibrillar proteins (Sleper *et al*., 1983) or increased water exudation (Scopes, 1964; Scopes, 1970), both of which will alter the reflectance of light from the surface of the muscle.

Previous studies have shown that meat colour darkens as intramuscular pH increases (Abril *et al*., 2001). Canada grade did not affect pH values, but ageing did affect pH and L* values. In the present study, steak aged 14 days had the highest mean pH, but the highest L* values. However, the pH difference was slight at only 0.02 pH units. Values of ultimate pH were negatively correlated (r = -0.42) to L* values, which may have indicated increased proteolysis as well (Scopes, 1964; Scopes, 1970). The lean of Canada A and AA LT muscles may be darker than that of Prime beef due an increase in the oxygen consumption rate by post-rigor beef muscle. The rate of oxygen consumption by beef is decreased by high early post mortem muscle temperature, and this contributes to an attractive bright red appearance of the exposed meat surface (Bendall *et al*., 1972).

Intramuscular fat is used to differentiate beef carcasses into the various quality grades and this differentiation is used as a measure of beef eating quality. Increased intramuscular fat has been found to improve sensory ratings of tenderness and juiciness (Miller *et al*., 1995; Wulf *et al*., 1997). In the present study, intramuscular fat as estimated by crude fat extraction increased with quality grade, with Canada Prime having the most intramuscular fat as expected. Unexpectedly, there was no difference in intramuscular fat content between Canada A and AA grades, suggesting that beef from the Canada A grade may be undervalued and that its level of intramuscular fat is not adequately detected by the naked eye. Quality grade also did not appear to be a reliable estimate cooked steak tenderness, as there was no difference between Canada Prime, Canada AAA, Canada AA and Canada A steaks, indicating that they were of comparable mean tenderness. There was a trend for Canada AA steaks to be the toughest, suggesting that cattle that grade Canada AA may be of a metabolic or physiological state not conducive to *post mortem* proteolysis. Cattle that are growing rapidly have been reported to have increased calpastatin (Sazili *et al*., 2004), and a high level of calpastatin decreases the proteolytic capacity of their meat early post mortem and reduces tenderization (Koohmaraie *et al*., 1995; Whipple *et al*., 1990). Although Canada AA carcasses produced the toughest *m. longissimus thoracis* steaks, the steaks may still be of acceptable eating quality as several authors (see section 1.4.1 tenderness) consider a steak to be tender at < 3.5 kg to <3.87 kg (Wulf *et al.,* 1998; Rodas-González *et al*., 2009). Steaks from Canada AA carcasses, with a WBSF value of about 4.0 kg would therefore be considered "acceptable" when using the tenderness thresholds of Wulf *et al*., (1998) or "tough" if using the criteria of Rodas-González *et al*., (2009). Although Canada A, AAA and Prime seem comparable in Warner-Bratzler shear force values, the additional fat of the Canada Prime and AAA

steaks may lead to a superior eating experience by improving beef flavour, juiciness and tenderness when assessed by consumer panels (Miller *et al*., 1995; Wulf *et al*., 1997).

The difference in toughness between 3 days aged and 14 days aged beef indicated by Warner-Bratzler shear force could be distinguished by an untrained consumer panel as previous studies have shown. Miller *et al*. (1995) and Huffman *et al*. (1996) reported that consumers could detect differences in tenderness between steaks when the difference in Warner-Bratzler shear force was equivalent to 1 kg. In the present study, the difference in Warner-Bratzler shear force between 3 days was and 14 days was by ~10 N (1 Kg.); therefore, ageing is worthwhile and strongly recommended. Although sensory analysis was not performed in this study, inclusion of sensory analysis in future studies is warranted for a complete assessment of sensory acceptability of beef from the various quality grades.

4.5 CONCLUSION

This study indicated that LT muscles from Canada Prime had meat quality characteristics different from those of the other grades, suggesting that Prime is appropriately valued as a superior product in markets that desire a high amount of fat in a steak or roast. Because Canada A and AA LT muscles had the lowest fat content of all the quality grades, beef marketing organizations could build a marketing strategy to direct this low fat beef to regions or countries where little fat in meat is desired. The lack of meat quality measurements difference among LT muscles graded Canada A, AA and

AAA suggested that LT from A and AA may be undervalued. Further research on the quality grades is needed to adequately describe the eating quality of the beef from these grades so that a complete quality profile of Canadian beef can be achieved. Because colour measurements are correlated to meat quality and especially fat, protein and moisture content, these should be taken into account when building an algorithm to predict meat quality. As well, establishing ideal or acceptable colour parameters may assist with prediction of beef eating quality beyond the amount of fat as long as strict protocols were developed to ensure measurement method consistency that standardized blooming time and meat oxygenation, instrument type and calibration, number of readings and measurement of lean tissue only.

4.6 TABLES

Table 4-1 Meat quality of high quality Canada beef grades

	CANADA GRADES					
Analysis	A	AA	AAA	PRIME	Pr > F[1]	SEM[2]
n	12	12	12	12		
Drip Loss (%)	1.19 [ab]	1.30 [b]	1.09 [a]	0.85 [c]	0.0011	0.13
WBSF[3] (Newton)	35.61	39.46	32.99	33.30	*0.0731*	2.83
Crude Fat (%)	2.13 [a]	2.63 [a]	4.96 [b]	11.11 [c]	<.0001	0.50
Protein (%)	22.90 [ab]	23.24 [a]	22.44 [b]	21.71 [c]	<.0001	0.18
Moisture (%)	73.59 [a]	72.73 [a]	71.48 [b]	65.59 [c]	<.0001	0.45

[1] Probability of the calculated F value with significance at $P \leq 0.05$

[2] SEM - Standard error of the mean

[a, b, c] Means with different superscripts within a row are significantly different at $P < 0.05$ according to least square mean differences tests.

[3] WBSF – Warner-Bratzler Shear Force

Table 4-2 Interaction effects of Canada beef grade and ageing on beef quality

	CANADA GRADES									
Analysis	A-3 days	A-14 days	AA-3 days	AA-14 days	AAA- 3 days	AAA-14 days	Prime-3 days	Prime-14 days	Pr > F[1]	SEM[2]
n	12	12	12	12	12	12	12	12		
a*	22.44 [ac]	23.02 [ab]	21.46 [c]	24.00 [bd]	23.40 [ab]	26.16 [ef]	25.37 [de]	27.30 [f]	0.0259	0.56
b*	6.21 [ac]	7.08 [b]	5.83 [c]	8.09 [de]	6.76 [abe]	8.76 [df]	7.43 [e]	9.64 [f]	0.0228	0.48
Cook Time (min/100g)	2.16	2.24	2.34	2.14	2.30	2.28	2.50	2.23	*0.0679*	0.09

[1] Probability of the calculated F value with significance at $P \leq 0.05$

[2] SEM - Standard error of the mean

[a, b, c, d, e, f] Means with different superscripts within a row are significantly different at $P < 0.05$ according to least square mean differences test

Table 4-3 Ageing effect on (*m. longissimus thoracis*) beef quality

		Ageing			
		3 days	14 days		
	n	48	48	Pr > F[1]	SEM[2]
Analysis	L*	37.43	38.17	0.0018	0.22
	Drip Loss (%)	1.17	1.05	*0.0781*	0.05
	Cook Loss (%)	17.62	18.99	0.0108	0.19
	pH	5.54	5.56	0.0328	0.00
	WBSF[3] (Newton)	40.34	30.35	<.0001	1.20
	Protein (%)	22.42	22.73	0.0139	0.10
	Moisture (%)	71.00	70.69	*0.0601*	0.28

[1] Probability of the calculated F value with significance at $P \leq 0.05$

[2] SEM - Standard error of the mean

[3] WBSF – Warner-Bratzler Shear Force

Table 4-4 Pearson Correlation coefficients among meat quality traits of beef *m. longissimus thoracis* (Rib Eye) at 3 days

Variable	L*	a*	b*	pH	Drip Loss (%)	Cook Loss (%)	WBSF[1] (Newton)	Cook Time (min/100g)	Fat Content (%)	Moisture Content (%)	Protein Content (%)
n	48	48	48	48	48	48	48	48	48	48	48
L*	1.00	-0.30*	0.37*	-0.42*	-0.00	0.28*	0.02	0.02	0.01	-0.02	-0.04
a*	-0.30*	1.00	0.66**	-0.08	-0.27	-0.26	-0.45*	0.18	0.67**	-0.61**	-0.53*
b*	0.37*	0.66**	1.00	-0.27	-0.28*	0.04	-0.34*	0.20	0.51*	-0.47*	-0.44*
pH	-0.42*	-0.08	-0.27	1.00	-0.10	-0.14	-0.01	-0.27	-0.22	0.26	-0.13
Drip Loss (%)	-0.00	-0.27	-0.28*	-0.10	1.00	0.07	0.15	-0.23	-0.39*	0.36*	0.14
Cook Loss (%)	0.28*	-0.26	0.04	-0.14	0.07	1.00	0.44*	-0.26	-0.31*	0.31*	0.05
WBSF (Newton)	0.02	-0.45*	-0.34*	-0.01	0.15	0.44*	1.00	-0.03	-0.28*	0.24	0.33*
Cook Time (min/100g)	0.02	0.18	0.20	-0.27	-0.23	-0.26	-0.03	1.00	0.31*	-0.34*	-0.02
Fat Content (%)	0.01	0.67**	0.51*	-0.22	-0.39*	-0.31*	-0.28*	0.31*	1.00	-0.98**	-0.59**
Moisture Content (%)	-0.02	-0.61**	-0.47*	0.26	0.36*	0.31*	0.24	-0.34*	-0.98**	1.00	0.51*
Protein Content (%)	-0.04	-0.53*	-0.44*	-0.13	0.14	0.05	0.33*	-0.02	-0.59**	0.51*	1.00

[1]WBSF – Warner-Bratzler Shear Force

* P=<0.05

** P=<0.0001

Table 4-5 Pearson Correlation coefficients among meat quality traits of beef *m. longissimus thoracis* (Rib Eye) at 14 days

Variable	L*	a*	b*	pH	Drip Loss (%)	Cook Loss (%)	WBSF[1] (Newton)	Cook Time (min/100 g)	Fat Content (%)	Moisture Content (%)	Protein Content (%)
n	47	47	47	48	48	48	48	48	48	48	48
L*	1.00	-0.29*	0.40*	-0.50*	0.08	0.22	0.11	0.05	0.15	-0.19	0.00
a*	-0.29*	1.00	0.68**	0.10	-0.35*	-0.36*	-0.24	0.16	0.63**	-0.58**	-0.49*
b*	0.40*	0.68**	1.00	-0.25	-0.19	-0.11	-0.04	0.26	0.60**	-0.60**	-0.43*
pH	-0.50*	0.10	-0.25	1.00	0.08	-0.02	-0.03	-0.25	-0.02	0.09	-0.21
Drip Loss (%)	0.08	-0.35*	-0.19	0.08	1.00	0.08	0.08	0.00	-0.55**	0.52*	0.37*
Cook Loss (%)	0.22	-0.36*	-0.11	-0.02	0.08	1.00	0.54**	-0.19	-0.27	0.26	0.14
WBSF (Newton)	0.11	-0.24	-0.04	-0.03	0.08	0.54**	1.00	0.01	-0.20	0.18	0.19
Cook Time (min/100g)	0.05	0.16	0.26	-0.25	0.00	-0.19	0.01	1.00	0.10	-0.16	-0.03
Fat Content (%)	0.15	0.63**	0.60**	-0.02	-0.55**	-0.27	-0.20	0.10	1.00	-0.97**	-0.68**
Moisture Content (%)	-0.19	-0.58**	-0.60**	0.09	0.52*	0.26	0.18	-0.16	-0.97**	1.00	0.59**
Protein Content (%)	0.00	-0.49*	-0.43*	-0.21	0.37*	0.14	0.19	-0.03	-0.68**	0.59**	1.00

[1]WBSF – Warner-Bratzler Shear Force

* P=<0.05

** P=<0.0001

Table 4-6 Pearson Correlation coefficients among selected meat quality traits of beef *m. longissimus thoracis* (Rib Eye) from 3 and 14 days ageing

Variable	L*-14 days	a*-14 days	b*-14 days	pH-14 days	Drip Loss-14 days (%)	Cook Loss-14 days (%)	WBSF[1]-14 days (Newton)	Cook Time-14 days (min/100 g)	Fat Content-14 days (%)	Moisture Content-14 days (%)	Protein Content-14 days (%)
n	95	95	95	96	96	96	96	96	96	96	96
L*-3 days	0.82**	-0.29	0.33	-0.51*	0.13	0.22	0.19	0.01	0.07	-0.08	0.01
a*-3 days	-0.14	0.68**	0.44*	0.14	-0.37	-0.24	-0.28	-0.00	0.63**	-0.57**	-0.52*
b*-3 days	0.47*	0.33	0.58**	-0.25	-0.15	0.03	-0.05	0.05	0.49*	-0.45*	-0.32
pH-3 days	-0.31	-0.18	-0.39	0.49*	0.25	-0.01	-0.13	-0.01	-0.26	0.30	0.01
Drip Loss -3 days (%)	0.00	-0.16	-0.05	-0.07	0.21	0.15	0.17	-0.12	-0.39	0.35	0.26
Cook Loss-3 days (%)	0.18	-0.26	-0.07	-0.27	0.09	0.34	0.04	0.02	-0.27	0.30	0.11
WBSF-3 days (Newton)	-0.03	-0.32	-0.24	-0.05	0.08	0.37	0.42	0.12	-0.27	0.20	0.34
Cook Time -3 days (min/100g)	0.12	0.07	0.11	-0.18	-0.32	0.02	0.17	0.28	-0.25	-0.31	-0.00
Fat Content-3 days (%)	0.15	0.62**	0.61**	-0.04	-0.57**	-0.31	-0.27	0.12	0.95**	-0.94**	-0.64**
Moisture Content-3 days (%)	-0.17	-0.58**	-0.60**	0.11	0.58**	0.27	0.24	-0.14	-0.93**	0.95**	0.56**
Protein Content-3 days (%)	-0.50	-0.35	-0.34	-0.13	0.23	-0.03	0.25	0.09	-0.56**	0.46*	0.59**

[1]WBSF – Warner-Bratzler Shear Force

* P= ≤0.0022

** P=<0.0001

Table 4-7 Regression models for Warner-Bratzler Shear Force Values (WBSF) of *m. longissimus thoracis* at 3 and 14 days ageing

Variable	WBSF[1]- 3 days	WBSF- 14 days	WBSF
1	a* (-1.88002)	Cooking Loss (1.10230	b* (-2.71900)
2	-	-	Cooking Loss (0.96723)
Model R-square	0.2092	0.2990	0.3016

[1] WBSF - Warner-Bratzler Shear Force

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Chapter 5

5.0 Meta-Analysis of Meat Quality Characteristics of M. *Longissimus Thoracis* (Rib Eye) from the Canada A, AA, AAA & Prime Grades

5.1 INTRODUCTION

Recently, two experiments were conducted to examine the meat quality characteristics associated with m. *longissimus thoracis* (LT, rib eye) according to Canada beef quality grade. The experiments were aimed at offering beef industry stakeholders a meat quality description of Canada's most marketed beef grades (Canada Prime, AAA, AA and A grades) so that carcasses could be differentiated and marketed based on a solid scientific meat quality description. Also, this research would assist with providing customers in Canada and around the globe with a consistent and known quality of the product.

In this chapter, the datasets from Chapters 2 and 4 were analyzed as one, with the main objective being to increase the experimental sample numbers from 12 to 24 LT steaks within each Canada grade to create a total of 96 steaks aged and 96 steaks non-aged steaks. The hypothesis of this study was that an increased number of samples will enable identification of differences in beef quality due to Canada grade. This hypothesis is based on the results of a previous study from the United

States on the influence of marbling on beef quality (McBee *et al*., 1967) where 8 different degrees of marbling and 40 carcasses per each degree of marbling were used for that study and differences were identified among the 8 degrees of marbling (for review, see Chapter 4, 4.1 Introduction).

5.2 MATERIALS AND METHODS

5.2.1 Experimental Design

Two experiments comparing the quality characteristics of beef *m. longissimus thoracis* (LT, rib eye) were performed over two years (2011-2012) and the results detailed in Chapters 2 and 4 of this thesis. Data included the following measurements performed on LT muscle aged 3 and 14 days: ultimate pH, CIE L*, a* and b*, drip loss, cooking loss, Warner-Bratzler shear force, and proximate composition (crude fat, moisture and protein). Methods used to estimate these characteristics were described in Chapter 2 (See Section 2.2 Materials and Methods). Data from these two experiments were combined and organized as a randomized complete block design, with year and block within year specified.

5.2.2 Statistical Analyses

The data were analyzed using the MIXED procedure in the Statistical Analysis System (SAS) software (Version 9.2, Statistical Analysis Systems, Cary, NC, USA). Analysis of variance was conducted using beef grade as the sole fixed effect and year and block (year) as random sources of variation, with block (year) as the error term for year. Data from non-

aged (3 days after retrieval from the abattoir) and aged (14 days after retrieval from the abattoir) beef were analyzed in separate data sets because roast position was not randomized in the first experiment. Grade effect was considered significant at $P \leq 0.05$ and where grade was significant least square mean differences were used to determine differences between grade means with significance at $P= \leq 0.05$. Least square means differences were separated by the PDIFF function of SAS. In all analyses of variance, degrees of freedom were corrected using the Kenward-Roger adjustment. Correlations analyses were performed using PROC CORR to obtain Pearson correlations among the meat quality data to identify possible linear dependence among variables. PROC REG was used to perform multiple regression and only non-correlated variables as determined using Pearson correlations were included in the model. Using simple linear regression, the inclusion of variables was dependent on influence of the variable on shear force values, and the stepwise model fit function was used.

5.3 RESULTS

5.3.1 Ultimate pH

No differences in ultimate pH due to Canada quality grade were noted at 3 and 14 days post mortem ($P = 0.2691$ and $P = 0.5793$, respectively) (Tables 5-1, 5-2).

5.3.2 Colour

5.3.2.1 L value*

At 3 days, differences in mean L* values between the Canada grades were found (P = 0.0235). Canada Prime had the highest L* values, with Canada AAA having the next highest mean L* value. Canada AAA had a higher mean L* value than that of Canada AA LT muscle but it was not higher than that of Canada A. No differences were found between the mean L* values of Canada AA and Canada A LT steaks (Table 5-1).

At 14 days, differences (P = 0.0217) among the Canada grades with regard to mean L* values were found. Canada Prime had the highest mean L* value that was significantly greater than that of LT from all other grades. There were no differences among the other grades for mean L* value (Table 5-2).

5.3.2.2 a value*

At 3 days, differences in a* values were found (P < 0.0001). Canada Prime had the highest mean a* value of all grades, while LT from Canada AAA carcasses had a higher mean a* value than that of Canada AA LT muscles but not higher than that of LT muscles from Canada A carcasses. No differences were found between LT muscles from Canada AA and Canada A carcasses (Table 5-1).

At 14 days, differences (P = 0.0217) in mean a* values between the Canada grades were found. LT muscles from Canada Prime carcasses had a higher mean a* value than that of LT muscles from the other grades

except for LT muscles from Canada AAA carcasses. Canada AAA carcasses had LT muscles with a higher mean a* value than that of LT muscles from Canada A carcasses, but not higher than that of LT muscles from Canada AA carcasses. No differences were found in mean a* values between LT muscles from Canada AA and Canada A carcasses (Table 5-2).

5.3.2.3 b value*

At 3 days, differences in mean b* values were found ($P < 0.0001$). Canada Prime LT had the highest mean b* value among all grades, with no differences in mean b* value among the other grades (Table 5-1).

At 14 days, differences ($P = 0.0013$) among mean b* values for the Canada grades were found. Canada Prime LT muscle had the highest mean b* value, with Canada AAA LT muscle had the next highest mean value, which was higher than that of LT muscles from Canada A carcasses, but not higher than those of Canada AA. Canada AA LT muscles had a higher mean value than those from Canada A carcasses (Table 5-2).

5.3.3 Drip Loss

At 3 days, drip loss differences among the grades ($P = 0.0105$) were found. Canada Prime had the lowest drip loss among the grades, with the exception of that from LT muscles of Canada AAA carcasses. Canada AA had a higher mean drip loss than LT from Canada AAA carcasses but not higher than LT from Canada A carcasses. No differences in LT drip loss were found between Canada A and Canada AAA carcasses (Table 5-1).

At 14 days, LT muscle drip loss differences were found between the grades (P = 0.0003). LT muscle from Canada Prime carcasses had the lowest mean drip loss among the grades, while LT from Canada AAA carcasses had a lower mean drip loss than those of Canada A carcasses. No other differences were found (Table 5-2).

5.3.4 Cook Loss

At 3 days, LT muscle cook loss differences (P = 0.0036) among grades were detected. LT muscles from Canada Prime had the lowest mean cook loss percentage, which was less than that observed for all other grades. No other differences were observed (Table 5-1).

At 14 days, LT muscle cook loss differences (P = 0.0036) among the grades were apparent. LT muscle from Canada Prime carcasses had a lower mean cook loss percentage than that of LT muscles from Canada AA and A grades. Also, LT muscles from Canada AA had a higher mean cook loss percentage than LT muscles from Canada AAA. No other differences were found (Table 5-2).

5.3.5 Cook Time

At 3 days, differences in cook time of the LT steaks between grades were found (P = 0.0016) with rib eye steaks from the Canada Prime grade taking longer to be cooked than those from all other grades(Table 5-1).

At 14 days, LT steaks from carcasses that graded Canada Prime still required a longer mean cook time to reach 71 °C than steaks from the other

grades, and there was a tendency for LT steaks from carcasses that graded Canada A to require the least amount of cooking time (Table 5-2).

5.3.6 Warner-Bratzler Shear Force

At 3 days, Warner-Bratzler shear force (WBSF) differences were found for the cooked LT steaks ($P = 0.0347$). Cooked LT steaks from carcasses that graded Canada Prime had the lowest WBSF values among the grades, with no other differences observed (Table 5-1).

At 14 days, Warner-Bratzler shear force (WBSF) differences were observed ($P < 0.0001$), with cooked LT steaks from Canada AAA carcasses having a lower mean WBSF value than those from Canada A & AA carcasses. Canada AA LT muscle had a higher mean WBSF value than LT muscle of carcasses from the other grades and no difference was found between LT muscles from carcasses that graded Canada Prime and Canada A (Table 5-2).

5.3.7 Proximate Analyses

5.3.7.1 Crude Fat Content

At 3 days ageing, differences in crude fat content were found among the Canada grades ($P < 0.0001$), with Canada Prime having the highest fat content followed by Canada AAA. No statistical difference was found between LT muscles from carcasses that graded Canada A and Canada AA (Table 5-3).

5.3.7.2 Protein Content

At 3 days ageing, differences were found in the protein content of LT muscles from the various Canada grades ($P < 0.0001$), with LT muscle from Canada Prime carcasses having the lowest protein content among the grades. LT muscle from Canada AAA carcasses had a lower mean protein content than LT muscle from Canada A and AA carcasses. No statistically significant differences were found between LT muscle from Canada A and AA carcasses (Table 5-3).

5.3.7.3 Moisture Content

At 3 days, differences were found for moisture content among the Canada grades ($P < 0.0001$), with LT muscle from Canada Prime carcasses having the lowest mean moisture content followed by LT muscles from carcasses that graded Canada AAA. There was no statistically significant difference in LT muscle mean moisture content between Canada A and Canada AA carcasses (Table 5-3).

At 14 days, differences among the Canada grades on moisture content were found ($P < 0.0001$), with Canada Prime having the lowest mean moisture content. Canada AAA had lower moisture content those of Canada A and Canada AA, but higher moisture content than those of Canada Prime. Canada A & Canada AA were not different from each other (Table 5-3).

5.3.8 Correlation among meat quality traits of beef m. *longissimus thoracis* (Rib Eye)

At 3 days significant correlations were found and are reported in Table 5-5. L* was correlated to b* (r = 0.44), pH (r = 0.22), cook time (r = 0.25), crude fat (r = 0.24), moisture (r = -0.20) and protein content (r = -0.27). a* was correlated to b* (r = 0.52), Warner-Bratzler shear force (r = -0.48), cook time (r = 0.25), crude fat (r = 0.42), moisture (r = -0.46) and protein (r = -0.27). b* was correlated to cook time (r = 0.31), crude fat (r = 0.39), moisture (r = -0.25) and protein (r = -0.52). Intramuscular ultimate pH was correlated to crude fat (r = 0.23) and protein (r = -0.42). Drip loss was correlated to cook loss (r = 0.21), crude fat (r = -0.30) and moisture (r = 0.26). Cook Loss was correlated to Warner-Bratzler shear force (r = 0.38), cook time (r = 0.33), crude fat (-0.35), moisture (r = 0.31) and protein (r = 0.20). Warner-Bratzler shear force was correlated to crude fat (r = -0.32), moisture (r = 0.30) and protein (r = 0.30). Cook time was correlated to crude fat (r = 0.37), moisture (r = -0.37) and protein (r = -0.29). Crude fat was correlated to moisture (r = -0.91) and protein (r = -0.58). Moisture was correlated to protein (r = 0.37).

At 14 days significant correlations were found and are reported in Table 5-6. L* was correlated to b* (r = 0.59), pH (r = -0.24), drip loss (r = 0.20), cook loss (r = 0.26) and cook time (r = 0.25). a* was correlated to b* (r = 0.70), pH (r = -0.23), Warner-Bratzler shear force (r = -0.48), cook time (r = 0.27), crude fat (r = 0.63), moisture (r = -0.21) and protein (r = -0.49). b* was correlated to pH (r = -0.26), cook loss (r = 0.27), Warner-Bratzler shear force (r = -0.31), cook time (r = 0.42), crude fat (r = 0.60), and protein (r = -0.43). Drip loss was correlated to cook loss (r = 0.26), Warner-Bratzler shear force (r = -0.31), crude fat (r = -0.55), moisture (r = 0.35) and protein (r = 0.37).

Cook loss was correlated to cook time (0.50) and moisture (r = 0.35). Cook time was correlated to crude fat (r = 0.49) and moisture (r = -0.23). Crude fat was correlated to moisture (r = -0.97) and protein (r = -0.68). Moisture was correlated to protein (r = 0.59).

5.3.9 Multiple regression

Table 5-7 shows results for multiple regression models. The multiple regression statistical analysis of the complete data with day 3 and 14 data combined produced a model (R^2 = 0.2768) to predict Warner-Bratzler values using a* values. Multiple regression analysis produced a model (R^2 = 0.2305) to predict Warner-Bratzler shear force at 3 days the model included the variables a* and cook loss. A model (R^2 = 0.2990) to predict Warner-Bratzler shear force at 14 days included in the model only cook loss. Multiple regression was used also to predict shear force values of each quality grade and predictive models were formulated for Canada A (R^2 = 0.1179), Canada AA (R^2 = 0.5510), Canada AAA (R^2 = 0.4797) and no variables met the requirement (P= <0.05) to compose a model for Warner-Bratzler shear force for Canada Prime.

Regression equations for Warner-Bratzler shear force for the combined day 3 and 14 data set and from the different ageing and grade treatments are as follow:

Overall (3 and 14 days) Warner-Bratzler shear force =

87.19172 -2.13571*(a*)

Warner-Bratzler shear force at 3 days=

61.96206 -2.01558*(a*) +1.40573*(cook loss)

Warner-Bratzler shear force at 14 days=

9.35171 -1.1023*(cook loss)

Warner-Bratzler shear force from Canada A=

13.17623 +11.17949*(cook time)

Warner-Bratzler shear force from Canada AA=

90.42923 -3.18148*(a*) +1.12429*(cook loss)

Warner-Bratzler shear force from Canada AAA=

118.94100 -3.41977*(a*)

5.4 DISCUSSION

As expected, at 3 days Canada Prime had the highest L*, a* and b* values, indicating that LT from this grade had a bright, rich red colour, and these traits in colour were maintained after 14 days ageing. These differences in colour may be related to the high amount of fat content of the Canada Prime LT muscle. Increased lightness of lean is related to warm muscle temperatures early post mortem (Bruce and Ball, 1990). As Canada Prime has high levels of marbling, this may be an indicator of increased subcutaneous fat as well, which could act as an insulator against the chiller low temperatures and keep the muscles warm early *post mortem* (Moon *et al*., 2006). The combination of high muscle temperature and low intramuscular pH early post mortem is capable of denaturing muscle proteins, which will increase the random configuration of the myofibrillar

proteins (Sleper et al., 1983) and increase intracellular water content and exudation (Scopes, 1964; Scopes, 1970). The combination of protein denaturation and increased free water may increase colour reflectance.

Pearson correlation results confirmed that colour is an important characteristic of beef. In the present experiment, a* values were negatively correlated to Warner-Bratzler shear force values at 3 and 14 days ageing (r = -0.48 and r = -0.48), indicating that as red colour increased the shear force value decreased. Also, L*, a* and b* values at 14 days were negatively correlated with ultimate pH values, indicating that a low ultimate pH was related to a light red beef colour. These findings agree with those of Abril *et al*., (2001), which showed that ultimate pH increased as the colour of the meat darkened. The results of the present experiment support the use of colour to predict the toughness of beef as colour had better correlation with shear force values than crude fat, and this finding agrees with that of Wulf *et al*., (1997), although these authors used marbling scores instead of chemical crude fat.

The differences in peak shear force between grades at 3 and 14 days ageing became significant with the meta-analysis as hypothesized. The present experiment confirmed the putative superior eating quality of LT from Canada Prime with Canada Prime being the most tender of steaks from all the Canada grades. Unexpectedly, there was no difference in WBSF among the other grades. These results imply that differences in tenderness among the other grades are not as stark and that valuation of these grades may be due to other characteristics not measured in the current study. Sensory analysis, although not performed in the present study, would elucidate any changes in flavour or juiciness between the

other grades, as these sensory characteristics can contribute to product differentiation. Differences in toughness between the other grades may also have been obscured by the unexpected increase in cooked LT toughness observed in the Canada AA grade. This increase carried through from the first experiment data set and is contrary to literature that shows that meat tenderizes over time after animal exsanguination (Koohmaraie *et al.,* 1987). Shear force values between day 3 and 14 of ageing in the first data set may have been compromised by changes in grill or materials testing machine function with time, inaccurate thermocouple placement within steaks, or lack of randomization between anterior and posterior portions of the LT muscle. Randomization between anterior and posterior locations within rib eye sections was not performed in the first experiment in order to accommodate requirements of another aspect of a parallel study. Janz et al. (2006) showed that toughness increased as steaks progressed from posterior to anterior on the LT, which is in agreement with the observed differences in the first experiment as muscle location was confounded with ageing time and anterior steaks were measured at 14 days ageing. Appropriate randomization occurred in the second experiment (Chapter 4) and Warner-Bratzler shear force was observed to decrease with ageing as expected.

Differences in cooking time among the grades were also found (Table 5-1; 5-2), and Canada Prime took longer to cook than the other grades. This may be related to its moisture and crude fat contents, as these two factors will influence the meat thermal conductivity greatly (Gavrilă *et al*., 2005). Gavrilă *et al*. (2005) reported that concentrated milk with high moisture content had a higher thermal conductivity than that with low moisture content. The same study by Gavrilă *et al*., (2005) also reported

that cream with high fat content had lower thermal conductivity than low fat cream. These results indicated that thermal conductivity increases with food moisture content and when fat displaces protein in meat it displaces its associated moisture, thus decreasing the thermal conductivity of the meat. With reduced thermal conductivity, cooking time to a specific temperature will therefore increase.

Again, as discussed in Chapter 4, colour indicators, particularly a* and b*, appeared to have the potential to predict Warner-Bratzler shear force. At 3 days, a* values appeared more useful to predict Warner-Bratzler shear force values than ultimate pH as the Pearson correlation between a* and WBSF was significant and that between Warner-Bratzler shear force and ultimate pH was not. At 14 days, a* and b* were both better predictors for Warner-Bratzler shear force values than ultimate pH. The b* value was found to be a good tenderness predictor in a study by Wulf *et al*., (1997), where the authors reported that b* was better than ultimate pH as tenderness predictor. Wulf *et al.* (1997) hypothesized that this occurred possibly because colour measurements had better repeatability or were better indicators of early post mortem pH decline than ultimate pH.

5.5 CONCLUSION

Differences were not easy to find among the Canada grades using objective measurements only and this may be related to the uniformity of the requirements needed to enter the Canada "A" grades. Once a carcass has qualified for the Canada "A" grades, the only differentiation between the various "A" grades is marbling score. Differences in tenderness and colour between grades may be related to the potential for high muscle temperatures to occur early post mortem that can cause increased proteolysis. Because shear values were negatively correlated with crude fat, this begs the hypothesis that meat with increased fat content ages faster than that with low fat. This was supported by the differences observed in shear force values between Canada Prime and the other grades were greatest at 3 days *post mortem*, and decreased with time as the other grades age as well. One more advantage of Canada Prime beef is that it could potentially be transported to market sooner than that from other grades as a guaranteed tender product because it was already more tender than the other grades at 3 days *post mortem*.

5.6 TABLES

Table 5-1 Means of meat quality characteristics for bovine LT muscle from high quality Canada grades at 3 days

	NON-AGED CANADA GRADES					
Analysis	A	AA	AAA	PRIME	Pr > F[1]	SEM[2]
n	24	24	24	24		
L*	35.17[a]	35.44[a]	34.76[a]	36.89[b]	0.0235	0.71
a*	20.25[b]	19.99[a]	21.23[b]	23.12[c]	<0.0001	0.70
b*	4.11[a]	4.17[a]	4.6[a]	6.22[b]	<0.0001	0.47
pH	5.36	5.36	5.39	5.43	0.26	0.03
Drip Loss %	0.58[a]	0.65[a]	0.46[ab]	0.29[b]	0.01	0.11
Cook loss %	17.17[a]	17.70[a]	16.61[a]	14.78[b]	0.0036	0.81
Cook Time (min/100gr)	2.1[a]	2.2[ab]	2.3[ab]	2.5[b]	0.0016	0.11
WBSF[3] (Newton)	41.84[a]	44.86[a]	41.74[a]	34.58[b]	0.0347	3.56

[1] Probability of the calculated F value with significance at $P \leq 0.05$

[2] SEM - Standard error of the mean

[3] WBSF - Warner-Bratzler Shear Force

[a, b, c] Means with different superscripts within a row are significantly different at $P < 0.05$ according to least square mean differences tests.

Table 5-2 Means of meat quality from high quality Canada grades at 14 days

Analysis	AGED CANADA GRADES					
	A	AA	AAA	PRIME	Pr > F[1]	SEM[2]
N	24	24	24	24		
L*	34.77[a]	34.70[a]	34.03[a]	36.56[b]	0.0217	0.84
a*	20.20[a]	21.45[ab]	22.62[b]	23.35[bc]	0.0027	0.86
b*	3.44[a]	4.58[b]	4.57[b]	5.72[c]	0.0013	0.55
pH	5.55	5.55	5.55	5.58	0.57	0.02
Drip Loss %	0.68[a]	0.62[ab]	0.54[b]	0.42[c]	0.0003	0.06
Cook loss %	16.05[a]	17.42[a]	15.02[a]	14.02[b]	0.0011	0.85
Cook Time (min/100gr)	1.8[ac]	2.1[bcd]	2.0[c]	2.3[d]	0.001	0.11
WBSF[3] (Newton)	44.83[a]	52.13[a]	39.98[bc]	42.15[c]	<0.0001	2.41

[1] Probability of the calculated F value with significance at $P \leq 0.05$

[2] SEM - Standard error of the mean

[3] WBSF - Warner-Bratzler Shear Force

[a, b, c] Means with different superscripts within a row are significantly different at $P < 0.05$ according to least square mean differences tests.

Table 5-3 Means of proximate analyses from high quality Canada grades at 3 days

		NON-AGED CANADA GRADES					
Analysis %	*n*	A	AA	AAA	PRIME	Pr > F[1]	SEM[2]
CRUDE FAT	96	2.60 [a]	2.85 [a]	4.78 [b]	11.39 [c]	<0.0001	0.4670
MOISTURE	96	72.40 [a]	72.08 [a]	70.68 [b]	65.48 [c]	<0.0001	0.5440
PROTEIN	96	23.70 [a]	23.81 [a]	23.17 [b]	22.10 [c]	<0.0001	0.2293

[1] Probability of the calculated F value with significance at $P \leq 0.05$

[2] SEM - Standard error of the mean

[a, b, c] Means with different superscripts within a row are significantly different at $P < 0.05$ according to least square mean differences tests.

Table 5-4 Means of proximate analyses from high quality Canada grades at 14 days

		AGED CANADA GRADES					
Analysis %	*n*	A	AA	AAA	PRIME	Pr > F[1]	SEM[2]
CRUDE FAT	48	2.15 [a]	2.44 [a]	4.89 [b]	11.23 [c]	<0.0001	0.7995
MOISTURE	96	71.43 [a]	71.13 [a]	69.89 [b]	64.47 [c]	<0.0001	0.5615
PROTEIN	48	23.15 [a]	23.54 [a]	22.45 [b]	21.79 [c]	<0.0001	0.3368

[1] Probability of the calculated F value with significance at $P \leq 0.05$

[2] SEM - Standard error of the mean

[a, b, c] Means with different superscripts within a row are significantly different at $P < 0.05$ according to least square mean differences tests.

Table 5-5 Pearson Correlation coefficients among meat quality traits of beef m. longissimus thoracis (Rib Eye) at 3 days

Variable	L*	a*	b*	pH	Drip Loss (%)	Cook Loss (%)	WBSF[1] (Newton)	Cook Time (min/100g)	Fat Content (%)	Moisture Content (%)	Protein Content (%)
n	96	96	96	96	96	96	96	96	96	96	96
L*	1.00	-0.05	0.44**	0.22*	0.15	0.10	-0.05	0.25*	0.24*	-0.20*	-0.27*
a*	-0.05	1.00	0.52**	0.15	0.05	-0.11	-0.48**	0.25*	0.42**	-0.36*	-0.39**
b*	0.44**	0.52**	1.00	0.16	-0.04	-0.09	-0.12	0.31*	0.39**	-0.25*	-0.52**
pH	0.22*	0.15	0.16	1.00	0.06	-0.15	-0.12	0.05	0.23*	-0.06	-0.42**
Drip Loss (%)	0.15	0.05	-0.04	0.06	1.00	0.21*	-0.03	0.04	-0.30*	0.26*	0.02
Cook Loss (%)	0.10	-0.11	-0.09	-0.15	0.21*	1.00	0.38*	0.33*	-0.35*	0.31*	0.20*
WBSF (Newton)	-0.05	-0.48**	-0.12	-0.12	-0.03	0.38*	1.00	0.19*	-0.32*	0.30*	0.30*
Cook Time (min/100g)	0.25*	0.25*	0.31*	0.05	0.04	0.33*	0.19	1.00	0.37*	-0.37*	-0.29*
Fat Content (%)	0.24*	0.42**	0.39**	0.23*	-0.30*	-0.35*	-0.32*	0.37*	1.00	-0.91**	-0.58**
Moisture Content (%)	-0.20*	-0.36*	-0.25*	-0.06	0.26*	0.31*	0.30*	-0.37*	-0.91**	1.00	0.37*
Protein Content (%)	-0.27*	-0.39**	-0.52**	-0.42**	0.02	0.20*	0.30*	-0.29*	-0.58**	0.37*	1.00

[1]WBSF – Warner-Bratzler Shear Force

* P=<0.05

** P=<0.0001

Table 5-6 Pearson Correlation coefficients among meat quality traits of beef m. longissimus thoracis (Rib Eye) at 14 days

Variable	L*	a*	b*	pH	Drip Loss (%)	Cook Loss (%)	WBSF[1] (Newton)	Cook Time (min/100g)	Fat Content (%)	Moisture Content (%)	Protein Content (%)
n	95	95	95	96	96	96	96	96	48	96	48
L*	1.00	0.17	0.59**	-0.24*	0.20*	0.26*	-0.17	0.25*	0.15	-0.10	0.00
a*	0.17	1.00	0.70**	-0.23*	0.10	0.08	-0.48**	0.27*	0.63**	-0.21*	-0.49*
b*	0.59**	0.70**	1.00	-0.26*	0.14	0.27*	-0.31*	0.42**	0.60**	-0.04	-0.43*
pH	-0.24*	-0.23*	-0.26*	1.00	-0.03	-0.08	0.07	-0.03	-0.02	-0.03	-0.21
Drip Loss (%)	0.20*	0.10	0.14	-0.03	1.00	0.26*	-0.31*	0.04	-0.55**	0.35*	0.37*
Cook Loss (%)	0.26*	0.08	0.27*	-0.08	0.26*	1.00	0.09	0.50**	-0.27	0.35*	0.14
WBSF (Newton)	-0.17	-0.48**	-0.31*	0.07	-0.31*	0.09	1.00	-0.06	-0.20	0.11	0.19
Cook Time (min/100g)	0.25*	0.27*	0.42**	-0.03	0.04	0.50**	-0.06	1.00	0.49*	-0.23*	-0.17
Fat Content (%)	0.15	0.63**	0.60**	-0.02	-0.55**	-0.27	-0.20	0.49*	1.00	-0.97**	-0.68**
Moisture Content (%)	-0.10	-0.21*	-0.04	-0.03	0.35*	0.35*	0.11	-0.23*	-0.97**	1.00	0.59**
Protein Content (%)	0.00	-0.49*	-0.43*	-0.21	0.37*	0.14	0.19	-0.17	-0.68**	0.59**	1.00

[1]WBSF – Warner-Bratzler Shear Force

* P=<0.05

** P=<0.0001

Table 5-7 Regression models for Warner-Bratzler Shear Force Values (WBSF) of m. longissimus thoracis including a classification by processing day and grade

Variable	WBSF[1]	WBSF- 3 days	WBSF- 14 days	WBSF- Canada A	WBSF- Canada AA	WBSF- Canada AAA	WBSF- Canada Prime[2]
1	a* (-2.13571)	a* (-2.01558)	Cook Loss (1.1023)	Cook Time (11.17949)	a* (-3.1814)	a* (-3.41977)	-
2		Cook Loss (1.40573)	-	-	Cook Loss (1.1242)	-	-
Model R-square	0.2768	0.2305	0.2990	0.1179	0.5510	0.4797	-

[1]WBSF – Warner Bratzler Shear Force.

[2]No variable met the P= <0.05 significance for entry the model.

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Chapter 6

6.0 Summary and Recommendations on Canada beef quality grades and suggestions for future Studies on Meat Quality and Early *Post Mortem* Carcass Management

6.1 RECOMMENDATIONS

6.1.1 Canada grades

Although the two experiments (Chapter 2 and 4) were performed by the same personnel using the same methods and equipment, there are several differences in data quality across the two experiments. One reason for data differences that may have influenced our study is that the meat supplier for the Chapter 2 experiment was different than that of Chapter 4, which may have positively influenced the database in terms of acquiring additional variation within the Canada quality grades. For the database of meat quality measurements of Canada grades to be representative, it is important that meat comes from different suppliers including other provinces. Even though Canada grades should be in theory all the same anywhere in Canada, this may not be the case as different processors have different processing practices, such as different chiller temperatures, different carcass management protocols. Also, electrical stimulation may or may not be used and even if it is used, its use can have significant

variations on voltage and amperage, which could have affected tenderness and colour greatly as demonstrated in Chapter 3.

In Chapter 4, although Canada AA carcasses produced the toughest *m. longissimus thoracis* steaks, the steaks may still be of acceptable eating quality as several authors (see section 1.4.1 tenderness) consider a steak to be tender at < 3.5 kg to <3.87 kg (Wulf *et al.*, 1998; Rodas-González *et al*., 2009). Steaks from Canada AA carcasses, with a WBSF value of about 4.0 kg would therefore be considered "acceptable" when using the tenderness thresholds of Wulf *et al*., (1998) or "tough" if using the criteria of Rodas-González *et al*., (2009). Although Canada A, AAA and Prime seem comparable in Warner-Bratzler shear force values, the additional fat of the Canada Prime and AAA steaks may lead to a superior eating experience by improving beef flavour, juiciness and tenderness when assessed by consumer panels (Miller *et al*., 1995; Wulf *et al*., 1997). The difference in toughness between 3 days aged and 14 days aged beef indicated by Warner-Bratzler shear force could be distinguished by an untrained consumer panel as previous studies have shown. Miller *et al*. (1995) and Huffman *et al*. (1996) reported that consumers could detect differences in tenderness between steaks when the difference in Warner-Bratzler shear force was equivalent to 1 kg. In the chapter 4 study, the difference in Warner-Bratzler shear force between 3 days was and 14 days was by ~10 N (1 Kg.). Therefore, ageing is worthwhile, effective and strongly recommended. Although sensory analysis was not performed in this study, inclusion of sensory analysis in future studies is warranted for a complete assessment of sensory acceptability of beef from the various quality grades.

When measuring the quality of meat objectively, no major differences can be found between grades, with the biggest difference between grades being crude fat content, as crude fat in the form of intramuscular fat is known to affect meat texture (Wood, 1990). This is not unexpected because intramuscular fat content is the basis for differentiating the various Canada quality grades. The results of Chapters 2 and 4 indicated that differentiation of grades based on marbling alone is not a guarantee to predict tenderness or other factors such as colour and ultimate pH because carcass temperature decline, carcass suspension methods, the use of hormone growth promotants, electrical stimulation, ageing and position of the steak in the primal may have a greater effect than marbling on beef tenderness. As a result, these factors should be considered when evaluating and pricing carcasses and cuts. Some of these factors are already been utilize by the AUS-MEAT language and the consumer grading system (Polkinghorne *et al*., 2010) in Australia. The purpose of this thesis is not to prompt change of the core of the current Canadian beef grading system, but to incorporate these factors as they have been identified to affect beef quality. Extending and maintaining the Canadian beef grading system as a voluntary program will keep all stakeholders competitive and profitable, with the advantage that having a guaranteed tender product will maintain and perhaps reverse the decline in per capita beef consumption as discussed in Chapter 1 (1.2.2 Meat Consumption Trends).

6.1.2 Future research on Canada grades

Further research on the quality grades is needed to adequately describe the eating quality of the beef from the four Canada quality grades so that a complete quality profile of Canadian beef can be achieved. Further studies have to be performed using trained and untrained tasting panels to evaluate meat quality traits such as texture, juiciness, flavor, colour and aroma of meat with different crude and cooked fat content, as these sensory characteristics can contribute to product differentiation.

As per objective measures, tenderness is affected by intramuscular connective tissue, myofibrillar proteins and their interactions (Sacks *et al*., 1998). Connective tissue is composed of collagen and elastin, but future research should focus on collagen because this protein is the principal component of connective tissue and fabricates the structural support for muscle and adipose tissue (Flint *et al*., 1984) and may affect beef tenderness. Further study of collagen and specifically heat soluble collagen, as its heat solubility is linked to meat toughness (Cross *et al*., 1973), will provide additional explanation as to how the chemical composition of Canada grades along with crude fat, moisture and protein contribute to beef tenderness.

In this study colour measurements were found to be correlated to shear force values especially a* (redness). Because colour measurements are also correlated to meat quality, especially fat, protein and moisture content, these should be taken into account when building an algorithm to predict meat quality. As well, establishing ideal or acceptable colour parameters may assist with prediction of beef eating quality beyond the amount of fat

as long as strict protocols were developed to ensure measurement method consistency that standardized blooming time and meat oxygenation, instrument type and calibration, number of readings and measurement of lean tissue only. Moreover, in recent years, visible and near-infrared spectroscopy (Chapter 1, Section 1.6.2.1) have demonstrated the potential to be used to predict meat toughness with an accuracy between 67% and 93%. This area of research may be worth additional investment as early results have been promising, particularly those using visible wavelengths (Leroy *et al*., 2003; Xia *et al*., 2007).

6.1.3 Future of carcass management early *post mortem*

In this study, electrical stimulation and chiller temperatures affected meat quality greatly, and as beef markets mature, the quality of beef may become more important than yield. To provide quality beef, Canada processors will need to have improved control of meat quality, and this could be accomplished by carcasses potentially being managed and grouped by weights and back fat thickness, so that a specific voltage and amperage can be provided to the carcass based on its fat coverage, weight and glucidic potential, together with a specific chiller regime to develop and reach its full ageing potential.

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