

Chapter 1: Introduction

The challenge of increasing efficiency during the slaughter process of beef and sheep meat is to maintain or enhance eating quality of meat. Although hot boning has been proven to be cost effective and accelerate processing time (Pisula & Tyburcy, 1996), it is often associated with inferior quality meat. As a result the adoption of hot or warm boning in the Australian sheep and beef meat processing industry has been limited to the use of adult sheep meat and aged cows and bulls. This process in most cases has been integrated with electrical stimulation to reduce the risk of muscle shortening due to the removal of the *pre-rigor* muscle from skeletal constraint (Devine, Hopkins, Hwang, Ferguson, & Richards, 2004).

It has been estimated in Australia that hot boning currently accounts for approximately 25% of Australia's mutton and goat production and 6-8% of beef production (C. Blaney, personal communication, May 19, 2011). New Zealand has largely pioneered the commercial application of hot boning in beef which accounts for approximately 20% of New Zealand beef production (Farouk, Wiklund, & Rosenvold, 2009). Traditionally, hot boned meat in New Zealand has been sourced from aged bulls and cows, but Simmons, Daly, Mudford, Richards, Jarvis, & Pleiter (2006) reported that this was no longer the case and it was now more common to process prime beef animals using hot boning. This change could be a result of the increasing demand for industry to not only reduce costs and increase energy efficiency but also reduce their environmental footprint.

The only published data on the eating quality of sheep meat processed through hot boning system within Australia shows a low level (~14%) of consumer satisfaction (Toohey & Hopkins, 2006a). Although there was some evidence that sarcomere shortening contributed to this result, sarcomere length (See Figure 2.2 for definition of

sarcomere length) only explained 10% of the variation in sensory assessed tenderness. In contrast when aged sheep were processed at the same abattoir, but conventionally chilled and cold boned, product aged for 7 days achieved a high level (86%) of consumer compliance (Hopkins, & Toohey 2006). In follow up work where loin meat was wrapped after hot boning and aged for seven days a 14% and 24% improvement respectively in the overall liking and tenderness scores was achieved compared to product unwrapped and frozen at 1 day (Toohey, Hopkins, & Lamb, 2008a). The improvement in shear force was more dramatic at 53%, where shear force is defined as the force required to slice through a cross-sectional area of cooked meat across muscle cells or fibres (Davey, 1983).

The concept of stretching or restraining *pre-rigor* muscles is not new. Since Locker (1960) discovered the relationship between sarcomere shortening and tenderness many studies have investigated possible techniques to increase sarcomere length and hence improve meat tenderness. These have included carcass interventions such as Tenderstretch (Hostetler, Landmann, Link, & Fitzhugh, 1970) and Tendercut (Wang, Claus, & Marriott, 1994) and individual muscle or cut interventions such as mechanical fixation and or stretch, muscle wrapping or Pi-Vac Elasto Pack System® (Troy, 2006).

The use of Tenderstretch in beef carcasses by Hwang, Park, Cho, Lee, Kim, Kim, & Thompson (2003b) showed the potential of this method for altering meat structure and thus improving eating quality in some cuts. Hopkins, Littlefield, & Thompson (2000b) reported that by super Tenderstretching lamb carcasses an even further reduction in shear force (26%) could be achieved in the loin compared with Tenderstretching and although some of the gain could be attributed to increased

sarcomere length and some to disruption of the I-band proteins (Hopkins, Garlick, & Thompson 2000a) the individual contribution of each effect is unknown.

Although significant meat quality benefits are shown when whole carcass interventions are applied, not all muscles can be improved (Troy, 2006). Locker (1960) concluded that in beef the degree of contraction when a muscle enters *rigor mortis* is highly variable among the different muscles of a carcass. Due to this variance between the different muscles/cuts, this poses another potential advantage of hot/warm boning, such that individual types of muscles/cuts can be manipulated differently in order to maximise meat tenderness and eating quality as opposed to conventionally chilled carcasses where all muscles are exposed to the same treatments.

In early work by Herring, Cassens, Suess, Brungardt, & Briskey (1967) where excised muscles were mechanically stretched using clamps this resulted in benefits in meat quality. Later Devine, Wahlgren, & Tornberg (1999) developed a technique in which they tightly wrapped beef *M. longissimus* using a polyethylene cling film. It was demonstrated that excising and wrapping meat could be used to control sarcomere shortening, but it could also be reduced by stretching muscle *pre-rigor*. Adopting the concept of super Tenderstretching by using weights (Hopkins et al., 2000a) and applying it to hot-boned beef muscle O'Sullivan, Korzeniowska, White, & Troy (2003) showed that equivalent tenderness could be achieved to that realised with the Pi-Vac Elasto Pack System®. Additionally the Pi-Vac Elasto Pack System® produced meat with the lowest variation indicating that this method does something different to meat structure.

These results suggest that if hot/warm boned meat is subjected to stimulation and then manipulated after boning that very high consumer compliance is possible. However under commercial hot boning processing conditions, given electrical

stimulation is often used to hasten the onset of *rigor* (Hwang, Devine & Hopkins, 2003a) to reduce the degree of muscle shortening when the muscles are removed from the skeletal constraint, it is critical that stretching occurs in *pre-rigor* muscles to ensure the benefits of stretching are not negated by rapid *rigor* onset. Efficiencies will be achieved if the manipulation overcomes the need for long ageing periods. Although success has been shown with previous techniques, there has been limited commercial adoption of these techniques. A new alternative stretching prototype device has been developed licensed as SmartStretch™ with the aim of manipulating hot-boned meat. The objective of the work described in this thesis was to investigate whether this alternative processing step (SmartStretch™) could maintain or enhance the meat quality of sheep and beef in commercial hot boning situations.

In order to understand how this alternative processing step of SmartStretch™ technology impacts on meat quality in particular tenderness, colour and water holding capacity, an understanding of muscle structure and changes that occur to muscle structure *post-mortem* is required. This is presented in the following literature review. Furthermore, a review of different factors that influence meat quality (production, pre-slaughter and in particular post-slaughter factors) is presented.

Chapter 2: Literature Review

2.1 Muscle

Skeletal muscle is an important component of meat and it is therefore important to be familiar with the composition and structure of muscle to fully understand how meat quality can be affected. Muscles are the contractile organ of the body with a complex structure.

2.1.1 Muscle composition

The composition of muscle tissue, after *rigor mortis*, but before *post-mortem* degradation changes, can be approximated to 75% water, 19% protein, 2.3% soluble, non-protein substances, 1.2% carbohydrates and 2.5% lipids (Olaoye, 2011). Muscle cells are among the most highly organized cells in the animal body and are required to perform a diverse array of mechanical functions. Thus the organisation, structure and metabolism of the muscle are keys to its function and to the maintenance of its integrity both during contraction and during the early *post-mortem* period (Huff Lonergan, Zhang, & Lonergan, 2010).

2.1.2 Muscle Structure

A schematic representation of muscle structure is illustrated in Figure 2.1. The whole muscle is surrounded by a layer of connective tissue called the epimysium. This whole muscle is comprised of bundles of fibres which are sheathed in connective tissue called the perimysium. Each muscle fibre is surrounded by its own connective tissue, the endomysium and by its own membrane, the sarcolemma (Tornberg, 1996).

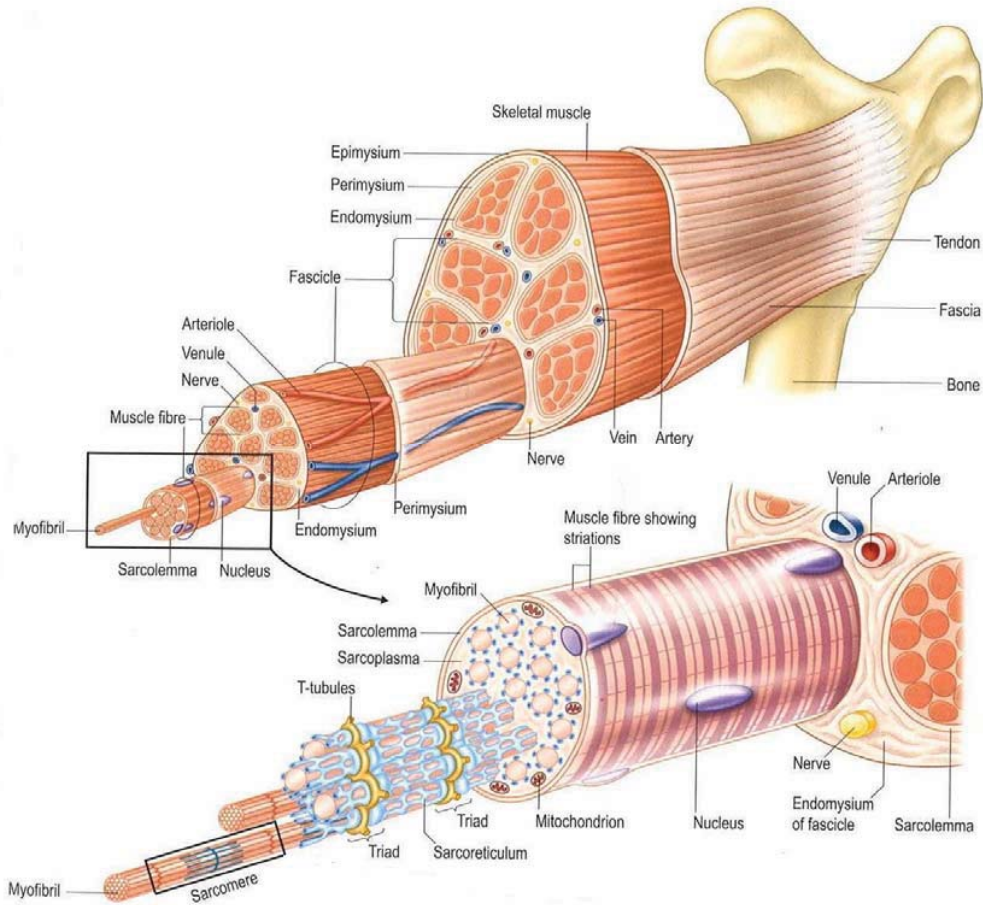


Figure 2.1. Microscopic structure of skeletal muscle (Adapted from; Aspinall, 2006).

Within each fibre are thousands of parallel myofibrils, the rod like organelles responsible for contraction, which occupy up to 80% of its volume (Tornberg, 1996). When examining a myofibril it can be seen that this cylindrical organelle is made up of repeating units which are known as sarcomeres (Huff Lonergan et al., 2010). The structure of the sarcomere is responsible for the striated appearance of the muscle (Figure 2.1). The myofibrils are bathed in the intracellular fluid known as the sarcoplasm, containing many sarcoplasmic proteins such as those responsible for glycolytic metabolism (Bechtel, 1986).

It was reported by Huff Lonergan et al. (2010) that recent proteomic analysis by Fraterman, Zeiger, Khurana, Wilm, & Rubinstein, (2007) indicated that over 65 proteins make up the structure of the sarcomere, although it was suggested by Huff Lonergan et al. (2010) that the actual number of proteins is likely to be far greater. The sarcoplasmic proteins constitute about 30-35% of the total muscle protein and the myofibrillar proteins (mainly the myosin and actin) which are the basis of thick and thin filaments respectively make up about 55-60% of total muscle protein (Tornberg, 1996). Given their prominence manipulation of actin and myosin will have a major influence on subsequent tenderness.

2.1.3 Sarcomere structure

Alternating light and dark bands that are visible under the microscope in the sarcomere (Figure 2.2) arise from the diffraction of light through the thick myosin and thin actin filaments. Thin filaments emanate from the Z-line and are in the I band while the thick and thin filaments make up the A band. The thin filaments extend into the A band while the H zone is the non overlapping region of the actin and myosin (Bechtel, 1986). Each sarcomere extends from one Z line to the next with the Z lines maintaining the spacing between the thin filaments (hence this is where sarcomere length is derived). The distance between the two Z lines or sarcomere length varies with the state of contraction of the muscle (Huxley & Hanson, 1954).

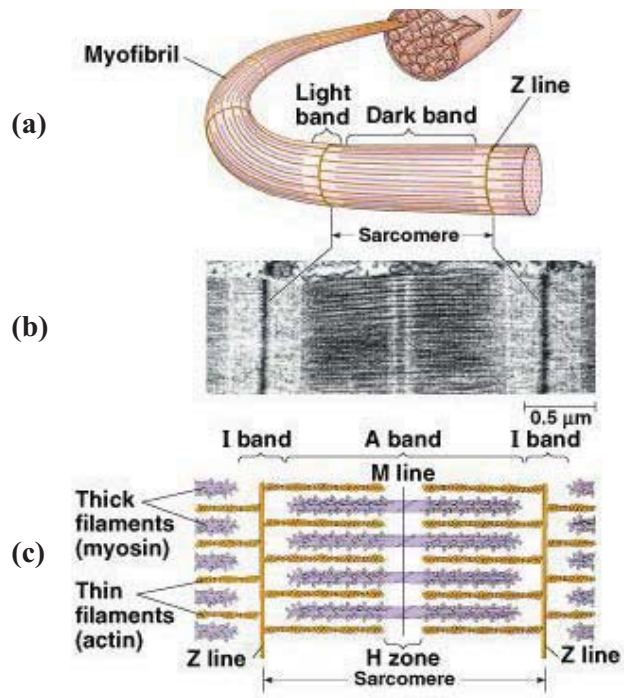


Figure 2.2. a) Schematic diagram of a single myofibril which is comprised of multiple sarcomeres, b) image from electron microscope showing the light and dark banding of the sarcomere structure and c) illustration of individual components of the sarcomere structure (Adapted from; Campbell & Reece, 2001).

Other myofibril proteins are responsible for maintaining the structural integrity of muscles via inter-myofibril linkages (desmin and vinculin) and intra-myofibril linkages (titin and nebulin) (Tornberg, 1996). Myosin consists of a tail or rod region that forms the main component of the thick filament and a globular head region that extends from the thick filament which interacts with actin in the thin filament. In order for contraction to occur, the thick and thin filaments interact via the head region of myosin. The complex formed by the interaction of myosin and actin is often referred to as actomyosin (Huff Lonergan et al., 2010).

2.1.4 Muscle contraction

Living muscle contracts through the overlapping of the actin and myosin filaments within myofibrils, upon nervous stimulation. In resting muscles, binding

between actin and myosin is prevented by the binding of tropomyosin and troponin to actin (Figure 2.3a). Upon stimulation due to ATP (adenosine triphosphate) depletion, calcium ions are released from the sarcoplasmic reticulum thus activating the glycolytic enzymes to produce ATP that provides the energy required for muscle contraction (Honikel, 2004). The myosin binding site on actin is exposed by calcium binding to troponin-C which moves troponin T aside so that myosin can bind with actin to form the actomyosin complex (Figure 2.3b). The charged myosin which is tightly attached to actin swivels and causes the filaments to slide (Jiang, 1995). Calcium ions are actively pumped back into the sarcoplasmic reticulum provided sufficient ATP is available, causing the actomyosin complex to break and the muscle to relax to its original state (Honikel, 2004). Contraction of *post-mortem* muscle occurs until the supply of ATP is depleted. The ATP depletion results in the actomyosin bonds becoming essentially permanent hence the muscle becomes stiff (Hwang, Devine & Hopkins, 2003a).

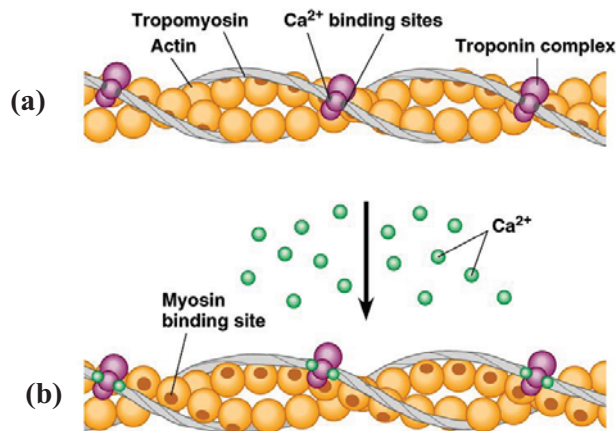


Figure 2.3. Association of troponin and tropomyosin with actin fibers. (a) Myosin binding sites blocked, preventing muscle from contracting (b) Myosin binding site exposed allowing muscle to contract (Source; Campbell & Reece, 2001)

2.2 Conversion of muscle to meat

The conversion from muscle to meat is a complex process and begins at death. When the heart stops beating the blood ceases to circulate which results in the depletion of muscle oxygen and a build up of cellular waste products (Honikel, 2004). The lack of oxygen in the muscle leads to a shift from aerobic production of energy to reactions that are anaerobic (Lawrie & Ledward, 2006). The breakdown of glycogen to produce ATP via anaerobic chemical pathways generates lactic acid which accumulates within the muscle. This causes the pH of the muscle to decline from a neutral pH of about 7.0 to an ultimate pH of around 5.5.

2.2.1 Rate of glycolysis

The rate of conversion of muscle to meat or glycolysis is dependent upon biochemical and physical characteristics of the muscle. The onset and extent of pH fall are biochemically characterised by the content of energy rich compounds including ATP, creatine phosphate and glycogen (Lawrie & Ledward, 2006). Factors that affect the rate of conversion of muscle to meat include species, breed, pre-slaughter conditions, muscle temperature, initial muscle glycogen content (nutrition) and muscle type (Aberle, Forrest, Gerrard, Mills, Hedrick, Judge, & Merkel, 2001). Post slaughter factors that affect the rate of glycolysis include electrical stimulation and rate of chilling. These factors will be discussed in greater detail in sections 2.5.1 and 2.9 respectively. Ultimately alterations to any of the conditions during *rigor* onset can alter ultimate meat quality (Bendall, 1973).

2.2.2 Changes in muscle structure during *rigor*

Rigor mortis is described by Honikel, Roncales, & Hamm (1983) as the irreversible loss of extensibility of a whole bundle of muscle cells. *Rigor* forms a stiff and inextensible state that is easily differentiated from the soft and workable state that appears *pre-rigor*. The changes in muscle extensibility are parallel to the changes in ATP content, thus as muscles begin to stiffen adenosine triphosphate (ATP) is depleting (Aberle et al., 2001). *Rigor* occurs in three phases that ultimately are dependant on temperature and the rate at which anaerobic glycolysis proceeds. The first phase of *rigor* is characterised by an extensible state of the muscle in which there is still sufficient amounts of ATP available to allow for relaxation of the muscle. During this phase, energy stores in the form of creatine phosphate, which allow for the phosphorylation of adenosine diphosphate (ADP) into ATP, are still sufficient to allow relaxation of the muscle. However, creatine phosphate is depleted during this stage which causes a sharp decrease in ATP production. The loss of extensibility and significant depletion of stored energy marks the beginning of the second phase or the onset phase. During the onset phase, muscles continue to increase in stiffness as more glycogen is depleted at the cellular level. This increase in stiffness continues until the third phase or the completion phase. The completion phase of *rigor mortis* is characterised by the almost complete loss of extensibility. Creatine phosphate is depleted and no ATP can be formed for relaxation, which results in no extensibility and full *rigor mortis* (Aberle et al., 2001).

Ultimately when the pH falls to around pH 6.0, the ATP level falls rapidly to a value which is insufficient to keep the actin and myosin apart thus they unite irreversibly to form inextensible actomyosin, producing the stiffness of *rigor mortis* (Hwang et al., 2003a). Both *rigor mortis* development and *post-mortem* pH decline is

dependant on energy utilisation. However, it is known that this does not occur across all muscle fibres simultaneously (Jeacocke, 1984) and as mentioned is dependent on initial glycogen levels (Hwang et al., 2003a). The rate at which a carcass or muscle is chilled has been shown to have a profound effect on energy utilization and ultimately tenderness (Hannula & Poulane, 2004; Honikel et al., 1983; Koohmaraie, 1996; Savell, Mueller, & Baird, 2005).

2.2.3 Muscle shortening

The chilling of carcasses within the first 24 hours *post-mortem*, when muscle is converted to meat, has been shown to be very critical with regards to meat tenderness (e.g. Geesink, Mareko, Morton, & Bickerstaffe, 2001; Honikel et al., 1983; Locker & Hagyard, 1963; Lochner, Kaufman & Marsh, 1980; King, Dikeman, Wheeler, Kastner & Koohmaraie, 2003). Numerous studies have identified that tenderness is improved if the temperature at *rigor* is some where between approximately 15-20°C (Devine, Payne, Peachey, Lowe, Ingram, & Cook, 2002a; Devine et al., 1999; Hertzman, Olsson, & Tornberg, 1993; Olsson, Hertzman, & Tornberg, 1994; Honikel et al., 1983; Hwang et al., 2003a; Locker & Hagyard, 1963; Nuss & Wolfe, 1980; Simmons, Singh, Dobbie, & Devine, 1996; Tornberg, 1996). Thompson, Hopkins, D'Souza, Walker, Baud, & Pethick (2005) predicted that in normally hung sheep carcasses the optimal temperature at pH 6 was 21 °C. These temperatures have been associated with minimum sarcomere shortening and maximum ageing potential. Hence if *rigor* occurs at low temperatures (often caused by rapid chilling of meat) this will result in cold shortening (Locker & Haygard, 1963). In addition, if *rigor* occurs at high temperatures this can lead to what is known as *rigor* or heat shortening.

2.2.3.1 Cold shortening

Cold shortening is a phenomenon that leads to the contraction and therefore shortening of sarcomeres of skeletal muscle due to a cold-induced release of calcium from the sarcoplasmic reticulum. Locker & Hagyard (1963) specified that the phenomenon of cold shortening occurs when muscle temperature declines to below 14°C before the onset phase of *rigor mortis* has started. The temperature and pH relationships at the moment of onset of *rigor* can be considered the most significant factors that influence the degree of cold shortening (Hannula & Puolanne, 2004). It is widely recognised that this contracture is ATP dependent and its extent will thus decrease as the muscle pH decreases (Honikel et al., 1983). When low muscle temperature occurs before the onset phase of *rigor*, the sarcoplasmic reticulum cannot function properly and is unable to absorb calcium ions, which leaves the sarcoplasm with a high concentration of calcium. Davey & Gilbert (1974) showed that there is an increase in the concentration of calcium ions in the myofibrillar region as the temperature drops from 15°C to 0°C and that, with an increase of free calcium ions, there is an increase in the shortening of the sarcomere. Because there is still ATP left in the muscle, muscle contraction occurs, causing the filaments to slide over each other, thereby shortening the sarcomere length (Figure 2.4).

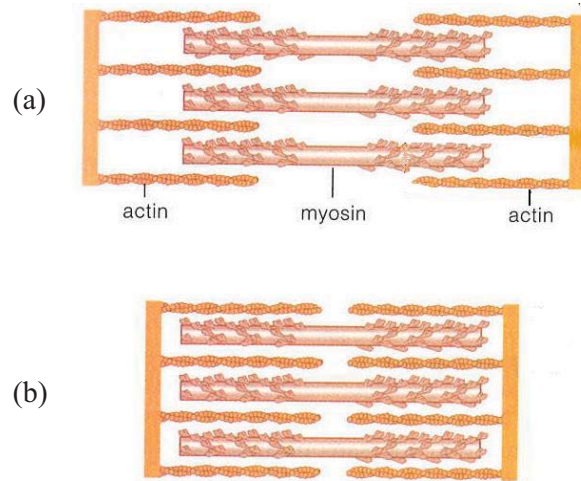


Figure 2.4. Simplified picture of muscle contraction a) muscle in relaxed state and b) muscle in contracted state (Adapted from; Starr & Taggart, 1998).

Ultimately the muscle shortening will become permanent due to the formation of the actomyosin bonds within the sarcomeres. According to Aberle et al. (2001), the sarcoplasmic reticulum is least functional at an internal temperature of 1-2°C. However muscle types vary in their potential to cold shorten, with red muscle fibres being more susceptible than white muscle fibres (Bendall, 1973).

2.2.3.2 Rigor (heat) shortening

In contrast to cold shortening, muscles that are maintained at higher temperatures for longer period *post-mortem* tend to have greater glycolytic rates, due to the increased activity of glycolytic enzymes and hence this causes a faster rate of decline in pH (Thompson, Perry, Daly, Gardner, Johnston, & Pethick, 2006). This condition leads to adverse meat-quality attributes, mostly due to the denaturation of muscle protein which gives rise to a pale colour and a low water-binding capacity similar to that normally associated with pale soft exudative (PSE) meat. The PSE condition can lead to shortening of the sarcomeres, which gives rise to the term *rigor* or heat shortening (Pike, Ringkob, Beekman, Koh, & Gerthoffer, 1993).

Rigor shortening occurs when muscle fibres are maintained at elevated temperatures resulting in a rapid depletion of ATP, followed by the myosin head binding irreversibly to actin and the early onset of *rigor*. High temperature shortening starts at a pH in the region of 6.2 and correlates with the time at which ATP begins to decline rapidly and continues until the end of glycolysis (Marsh, 1954). Due to the lack of ATP to energise the contraction it is far less severe than cold shortening (Thompson et al., 2006). However heat shortening can result in reduced ageing potential due to the denaturation of proteolytic enzymes (mainly μ -calpain). In general *rigor* or heat shortening appears to be a greater issue for the beef industry when compared to the sheep industry purely due to the greater size of beef carcasses and thus the retention of heat for a longer period *post-mortem*, hence a lower chilling rate.

2.2.3.3 Thaw shortening

Aberle et al. (2001) describes thaw shortening as occurring when *pre-rigor* muscle is frozen and then thawed. Under these conditions, contraction is caused by a sudden release of calcium from the sarcoplasmic reticulum. When *pre-rigor* muscle is frozen below about -20°C development of *rigor mortis* is halted. When this muscle is thawed at either chilled, ambient or elevated temperatures, *rigor* development (thaw *rigor*) can occur (Marsh & Thompson, 1958) which is accompanied by contracture or ‘thaw-shortening’ (Davey & Gilbert, 1973). This type of contracture is characterised by an irregular banding pattern of highly shortened sarcomeres (Cassens, Briskey, & Hoekstra, 1963).

2.2.3.4 Summary of muscle shortening

Locker & Hagyard (1963) reported that minimum shortening (10%) occurs at a temperature between 14°C and 19°C (Figure 2.5), which correlates with minimum meat toughness (Tornberg, 1996). Cold induced shortening can cause muscle to shorten up to 50% whereas when heat induced shortening occurs it is often less severe, but muscles can still shorten up to 30% (Locker & Hagyard, 1963). Under thaw *rigor* conditions muscles can contract up to 80% of their original length (Aberle et al., 2001).

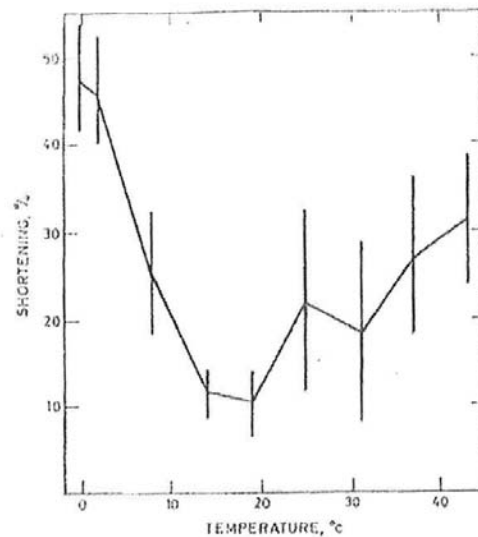


Figure 2.5. Mean shortening percent of muscles at various storage temperatures where the vertical line represent the standard deviation (source; Locker & Hagyard, 1963).

The temperature at which muscle shortens is dependent on species and muscle type. There is no way in which to completely prevent muscle shortening during the development of *rigor*. However, there are ways to reduce the extent and toughening effects of this process before, throughout and after slaughter (Savell et al., 2005). The *pre-rigor* temperature-pH environment and changes in muscle structure determine the quality attributes of tenderness, colour and water holding capacity.

2.3. Meat quality

Maltin, Balcerzak, Tilley, & Delday (2003) defined meat quality as a generic term used to describe properties and perceptions of meat. These attributes can range from carcass conformation and composition to food safety to eating quality. The visual appearance and overall eating quality are two main factors that have been identified to influence consumer choice to purchase and repurchase red meat (Maltin et al., 2003; Thompson, 2002; Egan, Ferguson, & Thompson, 2001). This review will focus on three key meat quality attributes including tenderness, colour and water holding capacity.

2.3.1 Meat tenderness

Meat tenderness is rated by consumers as the most important meat quality factor contributing to consumption. Meat tenderness is the main attribute that determines whether or not consumers will repurchase meat (Lorenzen, Miller, Taylor, Neely, Tatum, Wise, Buyck, Reagan, & Savell, 2003) and can thus directly impact on the growth and profitability of the meat industry. The Australian Beef Industry identified that variable eating quality was a major contributor to declining beef consumption in the early 1990s (Polkinghorne, Thompson, Watson, Gee, & Porter, 2008) and hence committed research funding to address this challenging problem. Given the influence tenderness has over consumers it is not surprising that one of the main goals worldwide is to produce meat of consistent tenderness to meet the requirements of today's more discerning consumer (Farouk et al., 2009).

As meat tenderness has been shown to be the most critical factor contributing to the overall eating experience it is considered to be the most researched meat quality trait. In general meat tenderness can be quantified in two ways either via subjective

consumer sensory testing or via objective mechanical testing, for instance shear force (Bouton, Ford, Harris, & Ratcliff, 1975). The relationship between objective and sensory measurements using Australian consumers of beef was examined by Perry, Thompson, Hwang, Butchers, & Egan (2001) and it was concluded that the objective measure of shear force was a useful indicator of sensory tenderness, however, it did not account for all variance in sensory testing particularly at the extremes when meat was really tender or tough. Most research examines meat tenderness via objective assessment. This is partly due to the high costs and logistics associated consumer sensory testing (Thompson, 2002). Objective meat tenderness has been defined as the amount of force required to slice a cross-sectional area of cooked meat across muscle cells or fibres (Davey, 1983).

In general a long sarcomere length predisposes muscles to greater tenderness and shortening of muscles induces toughening (Herring, Cassens, & Briskey, 1965; Marsh & Leet, 1966; Tornberg, 1996). Tenderness is also influenced by the content and quality of connective tissue and depends upon the age, sex, muscle type and species (Ouali, 1990). In the *pre-rigor* condition, muscle is tender and becomes tougher as *rigor mortis* sets in due to linkage of actin and myosin and then becomes more tender with ageing (Honikel, 2004) as shown in Figure 2.6. The extent of decrease in toughness with ageing seems to be related to the extent of the increase in toughness during the first 24 hours (Dransfield, 1994) due to alterations in sarcomere length and initial activity of the proteolytic enzymes. The mechanisms involved in tenderisation are both enzymatic and physiochemical in nature (Jiang, 1995).

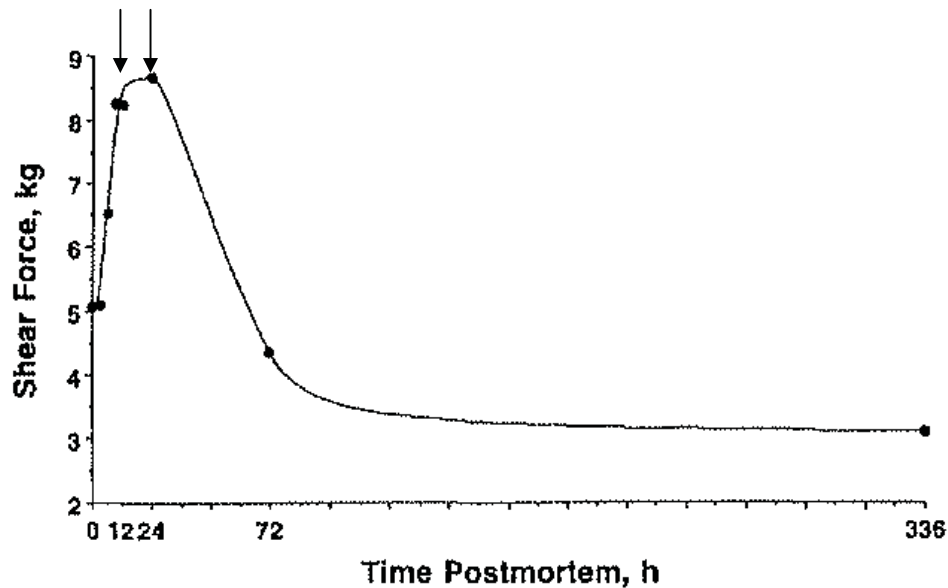


Figure 2.6. Proposed time course changes in shear force of ovine *M. longissimus* during *post-mortem* storage. Maximum contraction occurs somewhere between the arrows as the muscle enters *rigor* (Adapted from Wheeler & Koohmaraie, 1994).

The improvement in tenderness during ageing is largely due to proteolysis. Proteolysis results in a loss of structural integrity of the muscle (Koohmaraie & Geesink, 2006) with progressive fracturing of the thin filament attachments to the Z disc, but not at the cross bridges (Etherington, 1984). The differences in the rate and extent of post slaughter proteolysis are the major sources of variation in meat tenderness (Koohmaraie, 1996). The rate and extent of proteolysis is influenced by many factors such as the rate of glycolysis, temperature and genetic factors inherent to the animal (Dransfield, 1994).

A component of meat toughness is derived from the connective tissue, particularly that of the perimysium (Etherington, 1984). This element of toughness is dependent upon the amount of collagen and collagen cross linking and changes in stability of the links within the muscle as the individual fibres become increasingly cross linked with age (Purslow, 2005). When meat is cooked, the collagen fibres contract, increasing the muscle fibre density per unit area thereby increasing

toughness particularly in muscles with a high collagen content (Harper, Allingham, Whan, Commins, Drinkwater, Knight, Hunter, & Kurth, 1995). The factors that influence meat tenderness will be discussed in greater detail in sections 2.4-2.9.

2.3.2 Meat Colour

In a recent review paper by Mancini & Hunt (2005) it was stated that ‘meat purchasing decisions are influenced by colour more than any other quality factor because consumers use discoloration as an indicator of freshness and wholesomeness’. This view has been supported by other researchers such as Sanders, Morgan, Wulf, Tatum, Williams, & Smith (1997). If the consumer is dissatisfied with the meat colour it has a negative economic impact on the meat industry (Smith, Belk, Sofos, Tatum, & Williams, 2000).

Meat colour is greatly influenced by the rate and extent of glycolysis. The variation in meat colour is due to the myoglobin concentration of the muscle and changes in chemical state of myoglobin (Kropf, 2003). Aberle et al. (2001) reported that myoglobin contributes 80-90% of the total pigment in muscle tissue of a well bled carcass. Myoglobin is a water soluble metalloprotein that stores oxygen for aerobic metabolism in the muscle. It consists of a protein and a non-protein porphyrin ring with a central iron atom. The iron is a key factor in meat colour, but crucial factors are the oxidation state of the iron and which compounds are attached to the iron fraction of the molecule (Brewer, 2004).

Muscle tissue appearance is determined by the chemical state of the pigments (Figure 2.7). In fresh meat the myoglobin can exist in three forms: deoxymyoglobin the purple; the oxygenated oxymyoglobin bright cherry-red that consumers have been taught to expect and find attractive, and the oxidised metmyoglobin, greyish-brown

(Kropf, 2003). These three forms are continuously being inter-converted (van Laack & Smulders, 1990). In the absence of oxygen, the pigment is in the deoxymyoglobin state, which has dark purplish-red colour. When meat is first removed from deoxygenated environment (vacuum packaging or fresh surface is cut) on exposure to air, the meat pigment is rapidly oxygenated and oxygen binds to the iron ions to form oxymyoglobin. The depth and the rate of penetration are dependent on the rate of oxygen diffusion – the diffusion being deeper as the meat is exposed to oxygen for longer. Deoxymyoglobin and oxymyoglobin have the capability to be oxidised, which turns the pigment to a brown colour and yields metmyoglobin. The brownish layer forms under the oxymyoglobin and slowly starts to extend to the outside surface. The meat will thus discolour and become unacceptable to consumers (van Laack & Smulders, 1990). Jacob, D'Antuono, Smith, Pethick, & Warner (2007) reported that meat on retail display will begin to brown within 1-7 days. This browning is due to the formation of metmyoglobin and can be measured by the ratio of reflectance at 630 and 580 nm (Hunt, 1980). Khlilji, van de Ven, Lamb, Lanza, & Hopkins, (2010) found that consumers rated lamb loins unacceptably brown if the 630/580nm ratio falls below 3.3. The rate of metmyoglobin formation is influenced by both pre and post-slaughter conditions and hence the time meat can spend on retail display will vary. However Jacob et al. (2007) reported that retailers often discount meat after 2 days on display to avoid the detection of the browning colour change by consumers.

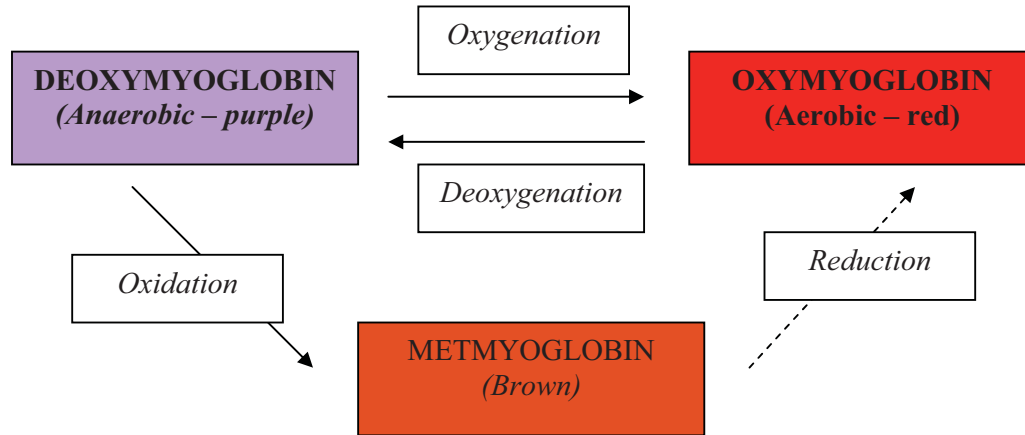


Figure 2.7. Principles of meat colour (Adapted from; Kropf, 2003)

Other influences that affect the perceived colour are physical and chemical factors such as pH, water-holding capacity and muscle structure (Lawrie & Ledward, 2006). The rate and extent to which muscle pH declines *post-mortem* impacts on meat colour. The normal pH decline in muscles is from approximately 7.0 - 7.2 down to near pH 5.5 - 5.7 over about 24 hours (Aberle et al., 2001). If there is a rapid decline in pH to 5.5 - 5.7 within roughly 45-60 minutes *post-mortem* thus at high carcass temperature, the muscle water holding capacity and colour intensity will decrease. This is caused by protein denaturation and the shrinking of the myofibrils resulting in expulsion of fluid from the muscle cells and drip loss. This enhances the light scattering power of meat and the meat becomes pale in appearance (Monin, 2004) and can be termed as pale, soft and exudative (PSE). In contrast if the pH does not drop noticeably *post-mortem* as a result of depleted glycogen levels at slaughter, the meat will be dark with a dull, dry surface (Monin, 2004) and can be termed dark, firm and dry (DFD). This darkening of colour becomes noticeable when the muscle pH exceeds 5.8.

The colour changes observed with PSE and DFD meat as indicated are mostly due to structural changes in muscle (Faustman & Cassens, 1990). In both cases, the spacing between filaments changes and when this spacing decreases, it increases the

extent to which light is reflected (because the spacing is closer to the wavelength of light) and this greater reflection of light means the colour is paler and less red. When the filament spacing increases, it decreases the extent to which light is reflected and a darker colour is shown (Conforth, 1994).

2.3.3 Water holding capacity

Water holding capacity of meat refers to the ability of meat to retain water. It too can be deemed as an important quality characteristic as it affects the appearance of meat before cooking, its behaviour during cooking and its juiciness during consumption (Huff-Lonergan & Lonergan, 2005). From a processing and retail perspective water holding capacity is seen as an important trait as increased water losses cause a decrease in final yield (Huff-Lonergan & Lonergan, 2005).

As muscle goes into *rigor* the water holding capacity of the muscle is reduced (Hamm, 1986; Honikel, 2004). Muscles consist of approximately 75% water (Olaoye, 2011) which is primarily bound by myofibrillar proteins with varying forces of interaction. The immobilisation of water is related to spatial arrangements of the proteins and the availability of intermolecular spaces between the myofibrils (Offer & Cousins, 1992) and most of the alterations in water holding capacity are due to changes in the physical configuration of the myofibrillar proteins. Muscle proteins increase in density with *rigor mortis* due to the binding of actin and myosin causing muscle contraction which reduces sarcomere length, thus the ability of the muscles to hold water is reduced due to the reduction in myofibrillar lattice space resulting in a loss of moisture (Honikel, 2004). This reduction in water holding capacity is further enhanced due to the lower water holding properties of actomyosin compared to myosin and actin alone (Offer & Knight, 1988). It was illustrated by Honikel, Kim, &

Hamm (1986) that there is a close relationship between muscle shortening (cold or heat) and percentage of drip loss, such that as a higher proportion of muscles shorten the percentage of drip loss increases.

The water holding capacity of meat is also reduced by the denaturation of both sarcoplasmic and myofibrillar proteins which occurs during *rigor* onset (Bond, Can, & Warner, 2004). Rapid pH decline while temperatures are still high denatures muscle proteins which results in a reduction of the water holding capacity (Offer, 1991). The changes in pH decline rate will influence the rate of myosin denaturation due to changes in the rate of actomyosin formation (van Laack, Kauffman, Sybesma, Smulders, Eikelenboom, & Pinheiro, 1994). Care must be taken during carcass processing to prevent conditions that will promote protein denaturation. When a rapid rate of reduction of muscle pH occurs post slaughter while carcass temperature is still relatively high, myofibrillar and sarcoplasmic proteins will be denatured, reducing the water holding capacity of the muscle and giving the muscle a paler colour (Honikel, 2004; Offer & Knight, 1988).

2.4 Factors that impact meat quality

There are many factors which influence meat quality in particular tenderness, colour and water holding capacity. Broadly these factors can be divided into three stages including production, pre-slaughter and post-slaughter factors.

Production factors that will impact on meat quality include; genetics (between breed and within breed variations) (e.g. in beef, Burrow, Moore, Johnston, Barendse, & Bindon, 2001; Johnston, Reverter, Ferguson, Thompson, & Burrow, 2003; in sheep, Hopkins & Fogarty, 1998; Safari, Fogarty, & Gilmour, 2005; Warner, Pethick, Greenwood, Ponnampalam, Banks, & Hopkins, 2007), animal temperament (Ferguson

& Warner, 2008), sex (Arsenos, Banos, Fortomaris, Katsaounis, Stamataris, & Tsaras, 2002), age (Bouton, Ford, Harris, Shorthose, Ratcliff, & Morgan, 1978a), nutrition and growth path (McKiernan & Wilkins, 2007).

Pre-slaughter factors include; animal handling, method of sale (Ferguson, Warner, Walker & Knee, 2007), transport (Wythes, Arthur, Thompson, Williams, & Bond (1981), changes in social structure and environment (Burrow & Dillon, 1997), pre-slaughter nutrition and fasting (Knee, Cummins, Walker, Kearney, & Warner, 2007), dehydration (Jacob, Pethick, Ponnampalam, Speijers, & Hopkins, 2006) and lairage time (Toohey & Hopkins, 2006b).

However despite many efforts to manage, control and optimise the pre-slaughter environment (Tatum, Belk, George, & Smith, 1999), which directly affects meat quality in particular tenderness (Ferguson, Bruce, Thompson, Egan, Perry & Shorthose, 2001), there is still unacceptable variation in meat quality suggesting there are many facets that determine meat quality and their interaction is complex (Maltin et al., 2003). In addition, even though as demonstrated, previous research has given meat producers the tools in theory to be able to deliver improved meat quality, there are often many factors that can prevent this from occurring. Some examples of these factors include, lack of adoption (either due to attitudes or financial constraints) and seasonal conditions, for example poor seasonal conditions may prevent animals from being finished off properly. Hence in order to reduce the variation in and or improve meat quality it is critical that post-slaughter factors are addressed.

It is generally agreed that *post-mortem* events have the greatest impact on meat quality in particular tenderness (Maltin et al., 2003). It was reported by Johnston, Reverter, Robinson, & Ferguson, (2001) that slaughter and subsequent carcass processing account for between 60-70% of tenderness variability, while contributions

from the animal itself and cooking make up the remaining 30-40 % in approximately equal amounts. Thus while other factors such as nutrition and genetics may be used as *in vivo* strategies to improve meat quality, these efforts may be in vain if the conversion of muscle to meat and other *post-mortem* factors are less than favourable (Maltin et al., 2003).

There are many post-slaughter factors which impact on meat quality including; whole carcass interventions (electrical stimulation, Tenderstretch, Tendercut and vascular infusion), hot boning, individual muscle or cut interventions (mechanical fixation and or stretch, muscle wrapping, Pi Vac, mechanical tenderisation, infusion, ultrasonic waves, hydrodynamic pressure, hydrostatic pressure and tumbling), chilling, and ageing. This literature review will focus on some of these post-slaughter factors with a particular focus on stretching interventions.

2.5 Whole carcass interventions to improve meat quality

2.5.1 Electrical Stimulation

The application of electrical stimulation is a useful tool which is now widely used in Australia, to accelerate tenderisation and improve meat quality traits. This concept is not new since the earliest recorded use of electrical stimulation was by Benjamin Franklin in 1749 where it was noted that electrocuted turkeys were ‘uncommonly tender’ (Devine et al., 2004). It wasn’t until 1951 when Harsham & Deatherage filed for a patent on electrical stimulation for tenderising of meat where electrical stimulation was used to accelerate the ageing of beef. However, the application of electrical stimulation in a commercial practise was not utilised seriously until 1970’s (Lawrie & Ledward, 2006), when first New Zealand and then Australia used it to avoid the toughness that results from cold-induced shortening (Hwang et al.,

2003a). This adoption of electrical stimulation technology can be attributed to extensive research throughout the 1970's into the effects it has on meat quality in particular meat tenderness (Carse, 1973; Chrystall & Hagyard, 1976; Chrystall & Devine, 1978; Davey & Gilbert, 1976; Cross, Smith, Kotula, & Muse, 1979; McKeith, Savell, & Smith, 1981a; Savell, Smith, Dutson, Carpenter, & Suter, 1977; Savell, Dutson, Smith, & Carpenter, 1978a; Savell, Smith, & Carpenter, 1978b).

The magnitude of the effects of electrical stimulation on meat quality for all species varies depending on the type, timing of the application *post-mortem*, voltage, frequency, duration of stimulation and method of application (Lee, Polidori, Kauffman, & Kim, 2000) as these things directly affect the rate of pH decline. Traditionally, there were two main forms of electrical stimulation employed, low voltage (e.g. Bouton, Ford, Harris, & Shaw, 1980; Eikelenboom, Smulders & Ruderus, 1985) and high voltage (McKeith, Smith, Savell, Dutson, Carpenter, & Hammons, 1981b; Toohey & Hopkins, 2006a). Often low voltage electrical stimulation is used in Australia to satisfy occupational health and safety regulations. More recently however, many studies have shown the influence of medium voltage stimulation on meat quality traits (Pearce, Hopkins, Toohey, Pethick, & Richards, 2006; Pearce, Hopkins, Williams, Jacob, Pethick, & Phillips, 2009; Shaw, Baud, Richards, Pethick, Walker, & Thompson, 2005; Toohey, Hopkins, Stanley, & Nielsen, 2008c).

Historically, electrical stimulation was aimed at preventing excessive muscle shortening (Swatland, 1981). Essentially, electrical stimulation acts by accelerating the onset of *rigor* (pH 6.0) resulting in a higher temperature at which a carcass enters *rigor* (Hwang et al., 2003a). When the muscles of freshly slaughtered animals are electrically stimulated, they contract. There is an associated increase in the

biochemical reactions in the muscle cells leading to an accumulation of lactate and resulting in an immediate drop in the muscle pH (Δ pH) (Devine et al., 2004). Electrical activation of the muscle membranes triggers calcium release from the sarcoplasmic reticulum, triggering the contractile actomyosin ATPase and muscle contractions. Muscle contractions result in a higher rate of ATP breakdown and this leads to a faster *post-mortem* glycolysis. The calcium ions also enhance the activity of phosphorylase, which provides the substrate for the increased rate of *post-mortem* glycolysis (Chrystall & Devine, 1985). The temperature at which a muscle reaches pH 6.0 and enters *rigor* can be used to predict meat quality (Thompson et al., 2005). If the carcass temperature falls too fast before the onset of *rigor* then as mentioned (section 2.2.3.1) cold shortening will result (Tornberg, 1996) which can have adverse effects on meat tenderness and other meat quality traits. Slow rates of cooling as mentioned (section 2.2.3.2) will lead to heat toughening (Devine et al., 1999) which may reduce ageing potential due to the denaturation of proteolytic enzymes such as μ -calpain (Strydom, Frylinck, & Smith, 2005).

In addition to the increased *post-mortem* glycolysis electrical stimulation is also thought to physically disrupt muscle fibres, and increase proteolytic degradation. Early work by Dutson, Yates, Smith, Carpenter, & Hostetler (1977) found that electrical stimulation resulted in changes in the ultrastructure of beef via physical disruption of muscle fibres. Histological images showed the appearance of contracture bands containing predominantly stretched ill-defined and disrupted sarcomeres as shown in Figure 2.8 (Hwang et al., 2003a). Since the study by Dutson et al. (1977) a number of other studies have also supported the hypothesis that electrical stimulation causes massive contraction which can in turn disrupt muscle fibres (Ho, Stromer, Rouse, & Robson, 1997; Savell et al., 1978a; Takahashi, Lochner, & Marsh, 1984;

Takahashi, Wang, Lochner, & Marsh, 1987) and hence increase tenderness. However there is some doubt regarding the importance of these contracture bands (Hwang et al., 2003a). Other studies have questioned whether the alterations in muscle structure are caused directly by electrical stimulation. George, Bendall, & Jones (1980) attributed the physical disruption to localised denaturation of sarcoplasmic proteins due to increased proteolytic activity. In a review on mechanical tenderizing mechanisms Hopkins (2004) also concluded that the contracture bands are not a direct result of electrical stimulation, but rather due to supercontracture caused by localised excessive release of calcium ions from the sarcoplasmic reticulum. The extra calcium released could directly lead to increased tenderness through the activation of enzymes Dransfield, Wakefield, & Parkman, 1992; Uytterhaegen, Claeys & Demeyer, 1992; Koohmaraire & Geesink, 2006).

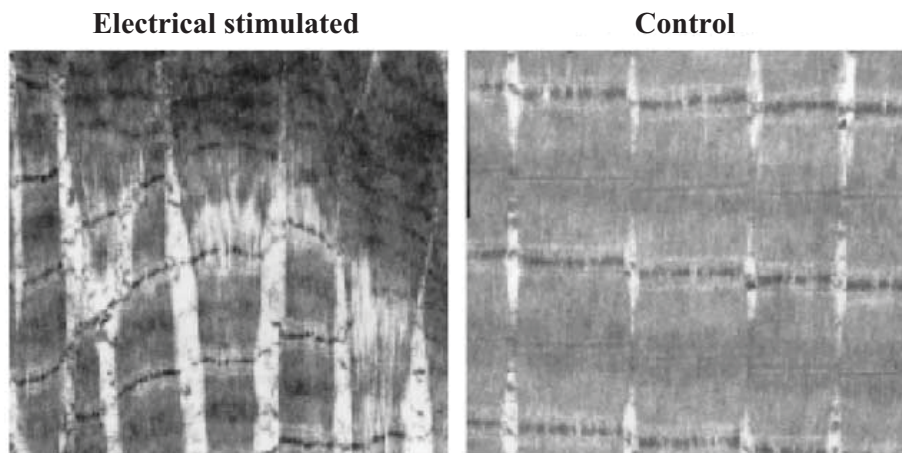


Figure 2.8. Characteristics of contracture bands in electrically stimulated beef *M. longissimus*, compared to controls after 1 day of ageing (Adapted from; Hwang & Thompson, 2002).

There have been many studies that have examined the effects of electrical stimulation on colour, colour stability and water holding capacity (e.g. Eikelenboom

et al., 1985; Moore & Young, 1991; Strydom et al., 2005; Toohey et al., 2008c). Again the effect electrical stimulation has on meat colour and water holding capacity is dependent on a number of factors such as the type of stimulation, timing of the application *post-mortem*, voltage, frequency, the duration of stimulation, method of application, muscle type, but importantly the chilling regime (Strydom et al., 2005). In general, electrical stimulation is associated with a higher *rigor* temperature and produces a paler colour, as noted by King, Voges, Hale, Waldron, Taylor, & Savell (2004), due to increased protein denaturation and myofibrillar lattice shrinkage (Offer & Trinick, 1983). The conditions of low pH and high temperatures in *post-mortem* muscle will also reduce the water holding capacity of meat and this effect is attributed to the denaturation of proteins, particularly myosin (Offer & Knight, 1988). Hence it is critical that the application of electrical stimulation is used in conjunction with effective chilling.

2.5.2 Tenderstretch

Locker (1960) suggested that there was a relationship between *post-rigor* sarcomere length and meat tenderness. Since this finding, many techniques have been developed to prevent *pre-rigor* muscles from contracting in order to improve meat tenderness. Tenderstretch is one method which was first reported by Hostetler et al., (1970). Tenderstretching is achieved by suspending carcasses from the aitch bone (*obturator foramen*) in split carcasses or the pelvis in whole carcasses as they come off the slaughter chain, hence placing increased tension on major leg muscles and loin muscles (Figure 2.9) before they pass through *rigor* (Thompson et al., 2005; Hopkins, 2004).

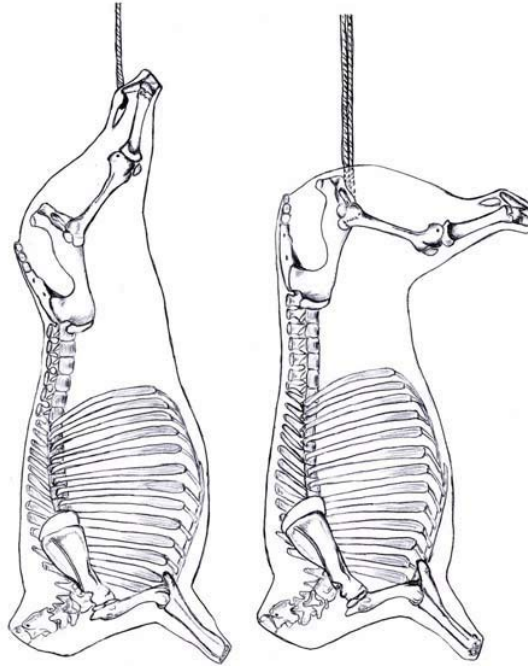


Figure 2.9. Schematic drawing of suspension methods. Carcase shape is affected by suspension from the achilles tendon (left) and Tenderstretch (Right).

As reported by Thompson (2002) the increased tension is aimed at either, stretching or minimising shortening (reducing the overlap of actin and myosin) of muscles and hence improving meat tenderness. The weight and the tension of the hind limb when it undertakes a more relaxed position leads to straightening of the vertebral column and prevention of muscle shortening due to skeletal restraint in both the back and the leg (Hostetler et al., 1970).

A number of studies across species have shown the positive effect of Tenderstretch in beef (e.g. Bouton & Harris, 1972; Hostetler et al., 1970; Hostetler, Link, Landman, & Fitzhugh, 1972; Hostetler, Carpenter, Smith, & Duston, 1975) and lamb or mutton (e.g. Bouton et al., 1973a; Quarrier, Carpenter, & Smith, 1972; Thompson et al., 2005). Based on these studies, in general it can be concluded that the application of Tenderstretch is able to increase sarcomere length and thus improve

meat tenderness of major hind leg muscles including, *Mm. semimembranosus*, *gluteus medius*, *vastus lateralis*) and loin (*M. longissimus*) muscles.

However the notable exceptions to this improvement in the hind leg are *Mm. biceps femoris* and *semitendinosus*. The *M. semitendinosus* is actually stretched more when carcasses are hung by the Achilles tendon. In addition to this the *M. psoas major* is actually allowed to shorten when the Tenderstretch method is applied. The muscles of the forequarter are not improved in tenderness with the application of Tenderstretch as no extra stretch or weight is applied to this part of the carcass (Sørheim & Hildrum, 2002).

Tenderstretching has not only shown to improve tenderness of key muscles, but it has also shown to reduce the variation in tenderness (Sørheim, Idland, Halvorsen, Frøystein, Lea, & Hildrum, 2001). In addition, other studies have shown that Tenderstretching can improve water holding capacity (Bouton et al., 1973a).

Hopkins et al. (2000a) reported that by super Tenderstretching lamb carcasses (by weighting the hind legs) even further reductions in shear force (26%) could be achieved in the loin compared with Tenderstretching. Although some of the gain could be attributed to increased sarcomere length and some to disruption of the I-band proteins (Hopkins et al., 2000b), the individual contribution of each effect is unknown. It was found that super Tenderstretching did not reduce shear force in the topside compared with Tenderstretching or produce a significantly longer sarcomere length (Hopkins et al., 2000a).

The practical implications to the adoption of this technique have included the need to alter the slaughter line in order to alter the hanging position of the side or carcass. Perhaps one of the greater disadvantages is that with the Tenderstretched hind leg hanging at a 90° angle there is a requirement for additional chilling floor space

(Sørheim & Hildrum, 2002). In addition as previously highlighted this technique is unable to improve all muscles. Commercially the adoption of this technique within Australia has been greater in the beef industry than in the sheep industry.

2.5.3 Tendercut

Another *pre-rigor* carcass muscle stretching treatment known as “Tendercut” was first developed in the USA in the early 1990’s by Claus and Marriott from the Virginia Polytechnic Institute and State University (Claus, Wang, & Marriott, 1997). This technique involves severing of bones and connective tissue to enable the weight of the carcass to stretch selected muscles prior to the onset of *rigor* (Wang et al., 1994) whilst the carcass is still suspended by the Achilles tendon. Cuts can be strategically located anywhere, but normally one cut is placed between the 12th and 13th thoracic vertebrae (Figure 2.10). Muscles adjacent to the *longissimus* such as the *multifidus dorsi* are also severed. The second cut severs the ischium of the pelvic bone and the junction between the 4th and 5th sacral vertebrae with adjacent connective tissues at the round and loin region (Wang et al., 1994) and this maximises the stretch on the *M. longissimus* (Figure 2.10).

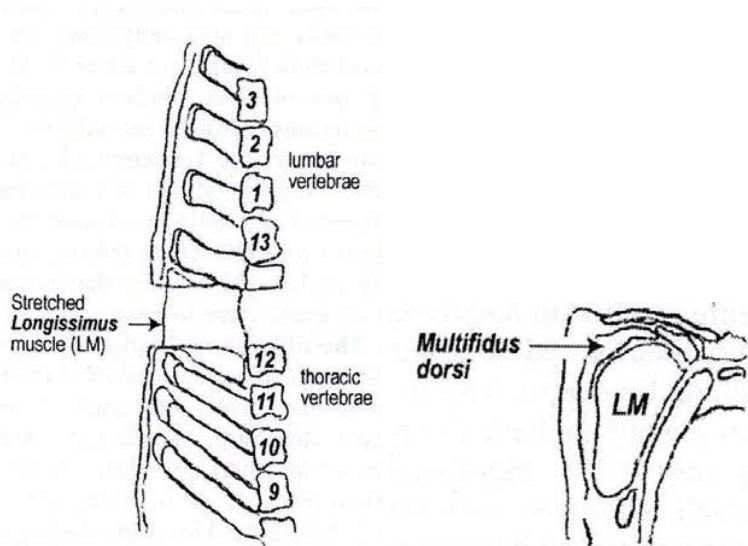


Figure 2.10. Schematic drawing of the TenderCut system with a cut in the 12th/13th vertebral region of a beef carcass side. A cross section of the *longissimus* muscle (LM) is shown to the right (Claus, 1997).

The aim of Tendercut technique is similar to that of Tenderstretch which is to increase tenderness in the *M. longissimus* and in the hindquarter muscles by increasing sarcomere length through stretching and by preventing sarcomere shortening during *rigor* (Wang et al., 1994). The major benefit of Tendercut is that the process is able to be incorporated into existing meat processing chains without altering equipment (Claus et al., 1997; Hopkins, 2004; Ludwig, Claus, Marriott, Johnson, & Wang, 1997). Gaps of significant size should appear in the cutting areas to ensure sufficient stretching effects (Sørheim & Hildrum, 2002). In addition to the early work in USA modified versions of the Tendercut method have been validated by both Canadian and Norwegian researchers (Aalhus, Best, Costello, & Jeremiah, 1999; Sørheim et al., 2001).

Research on the benefits of Tendercut has produced variable tenderness results. Many studies including those of Claus et al. (1997), Ludwig et al. (1997) and Wang et al. (1994) confirm the benefits of Tendercut in increasing the tenderness of beef loin and round muscle. However Beaty, Apple, Rakes, & Kreider, (1999) found that by

using the Tendercut process in lightweight heifer carcass sides the sarcomere length of both the loin and round increased compared to the control treatment, but tenderness only improved in the loin. Hence while research has shown an increase in sarcomere length resulting from the Tendercut process this was not always matched with a commensurate improvement in sensory response or in shear force results (Beatty et al., 1999; Claus et al., 1997; Ludwig et al., 1997; Shanks, Wulf, Reuter, & Maddock, 2002; Wang, Claus, & Marriott, 1996). In the study by Sørheim et al. (2001) Tendercut was found to only have a favourable impact on shear force and sensory results if the carcasses were rapidly chilled. However it was reported by both Claus et al. (1997) and Sørheim et al. (2001) that Tendercut was beneficial in reducing the variation in sensory tenderness in beef *longissimus* muscles.

Research into other meat traits such as cooking loss and thaw loss (indication of water holding capacity) and meat colour have also been examined. Tendercut has no effect on cooking loss (Claus et al., 1997; Ludwig et al., 1997; Wang et al., 1996) or thaw loss (Wang et al., 1996). These results are contrasted to other studies where stretched muscles have been found to have greater water holding capacity when compared to control (e.g. Bouton et al., 1973a). Some studies have indicated that meat colour was unaffected by Tendercut (Ludwig et al., 1997; Wang et al., 1996), while other studies showed a significant reduction in redness (Claus et al., 1997).

It seems that based on the published literature the Tendercut technique does not achieve the same degree of reduction in shear force in the loin and leg muscles as Tenderstretching, although it has been shown to reduce the variation in tenderness (Claus et al., 1997; Sørheim et al., 2001). Additional implications of the Tendercut technique reported by Sørheim & Hildrum, (2002) are that making the two cuts on each side is more time consuming than application of the Tenderstretch technique, and

to be effective, are dependent on well defined criteria for specific cutting. Given that carcasses are still hung from the Achilles tendon they do not require any extra floor space, but due to the extra cuts the sides can be extended in length by 15-20cm which needs to be considered with regard to rail height (Sørheim & Hildrum, 2002). The method would not be applicable to Australian sheep processing systems given the requirement for *pre-rigor* splitting of the carcass. In addition, as previously highlighted with Tenderstretch, the Tendercut technique is also restricted by the muscles it can improve.

2.5.4 Summary

Although significant meat quality benefits are shown when whole carcass interventions are applied, one of the main issues with this approach is the non-uniformity of muscles on a carcass (Troy, 2006). The ability to apply additional processing intervention techniques directly to individual muscles has the potential to improve meat quality traits according to the individual muscle needs. Hot boning is one method which can facilitate this concept of treating muscles individually.

2.6 Hot boning

Hot-boning can be defined as the removal of muscle from the carcass prior to the completion of *rigor mortis* (Devine et al., 2004). The concept of deboning meat from “hot” carcasses was first put forward in the early 1970’s with the advantages cited such as reduced chiller space, labour cost and better yields (Cuthbertson, 1979) and was originally developed in response to commercial demands to essentially lower energy usage and costs (Troy, 2006).

Many studies (Jeremiah, Martin, & Murray 1985; McPhail, 1995; Pisula & Tyburcy, 1996; Waylan & Kastner, 2004) have highlighted the benefits associated

with hot-boning. These benefits include: reduction in weight loss during chilling (~1.5%); reduction in drip loss during storage (~0.1-0.6% depending on muscle and chilling parameters), hence higher final yields; reduction in chiller space (~50-55%), energy saving in inputs on refrigeration (~40-50%); faster turnover of meat in the processing plant (~40-50%); reduction in capital costs for buildings; and finally savings in both labour (~20%) and transport. In addition to these benefits it has been reported that because warm meat is softer it requires less effort to bone and hence occupational overuse injuries are less likely to occur (Anonymous, 2001). More recently White, O'Sullivan, Troy, & O'Neil (2006) raised an additional benefit of hot boning and that is the ability to separate carcasses during the critical early *post-mortem* period and treat individual primals or cuts according to their intrinsic properties. This supports the conclusions of work by Locker (1960) where it was found in beef that the degree of contraction when a muscle enters *rigor mortis* is highly variable among the different muscles of a carcass. Hot boning gives the opportunity to manipulate shape and stretch of individual muscles, given muscles are in a *pre-rigor* state and the sarcomeres are not fixed by actomyosin bonds and are able to be extended by forms of mechanical interventions (Simmons et al., 2006).

Despite the many advantages of hot boning there are major constraints. Pisula & Tyburcy (1996) described the limitations of existing plants being able to adopt the technology due to having to make significant changes to their current processing systems (for example the need to synchronise the slaughter and boning processing systems). Spooncer (1993) outlined the increased risk of bacterial growth if optimal drying and cooling systems were not used. Hot boning also results in increased shape distortion of cuts and muscles can become separated (Pisula & Tyburcy, 1996; Troy, 2006) this can create issues when slicing (McPhail, 1995). In the case of beef,

traditional carcass grading (assessment of colour, marbling & pH) is also difficult (McPhail, 1995).

The impact that hot boning has on meat tenderness is a significant concern with regard to producing quality meat. Hot boning increases the risk of muscle shortening (Devine et al., 2004) due to the removal of the *pre-rigor* muscle from skeletal constraint (Locker, 1960) leading to subsequent shortening which is a major influence on meat tenderness (Tornberg, 1996). Hence hot boning is usually performed in conjunction with electrical stimulation. The only published data on the eating quality of sheep meat processed through this system shows a low level of consumer compliance with only ~14% of samples tested achieving the ‘good everyday’ requirement (Toohey & Hopkins, 2006a). In contrast, when adult sheep were processed at the same abattoir, but conventionally chilled and cold boned, product aged for 7 days achieved a high level of consumer compliance with 86% of samples tested achieving a ‘good everyday’ ranking (Hopkins & Toohey, 2006). Hence although this shortening can be minimised through the use of electrical stimulation, in order to further improve the tenderness of hot-boned meat another approach to prevent muscle shortening is to restrain the muscles physically until they are in *rigor mortis* (Macfarlane, Harris, & Shorthose, 1974).

In general the colour of hot-boned muscles has been shown to be slightly darker, but more uniform than that of conventionally chilled carcasses (Brown, Gigiel, Swain, & Higgins, 1988), however, the increased darkness in colour is generally not deemed as unacceptable (Waylan & Kastener, 2004). It is thought that the darker colour exhibited by hot-boned meat is associated with faster cooling rates and more intensive respiratory action (Renner, 1990). This result is largely a function of the process of hot boning, given that hot-boned meat is often directly vacuum packed and thus

placed in a oxygen free environment early which will limit metmyoglobin formation (browning of meat) (Knopf, 2003). Although there have been contrasting results on the impact of hot boning on water holding capacity it can be concluded that under optimal chilling conditions it does not appear that hot boning has any impact on water holding capacity (Babiker & Lawrie, 1983; van Laack & Smulders, 1992).

The adoption of hot or warm boning in the Australian sheep and beef meat processing industry has been limited to the use of adult sheep meat and aged cows and bulls. This process in most cases has been integrated with electrical stimulation. Traditionally, hot-boned meat in New Zealand has been sourced from bulls and cows, but Simmons et al. (2006) reported that this was no longer the case and it was now more common to process prime beef animals using hot boning. It was also reported that regular audits of product quality have demonstrated that eating quality and other quality attributes can match those of meat produced by cold boning procedures, if pH and temperature decline were effectively managed (Simmons et al., 2006). It should be noted that there was no evidence presented to support these latter claims. Results from work by Toohey & Hopkins (2006a) indicated that even with the application of electrical stimulation other processing interventions would be required to achieve acceptable eating quality by Australian standards, given only 14% of sensory samples were considered acceptable. Thus it would appear questionable whether quality could be maintained solely by managing pH and temperature decline without having an increased risk of bacterial growth (Sponcer, 1993).

2.7 Individual muscle or cut

2.7.1 Mechanical stretching techniques

When muscles are removed from their skeletal restraint *pre-rigor* the chance of muscle shortening is increased dramatically. The research by Locker (1960) demonstrated the relationship between myofibrillar shortening and meat tenderness, which was supported by Herring et al. (1965). In order to examine the effects of restraining or stretching muscles various clamping devices (Figure 2.11) were developed for research purposes which enabled muscle to be stretched to a certain length (Bruce & Ball, 1990; Buege & Stouffer, 1974; Herring et al., 1967; Locker & Leet, 1975; Marsh & Leet, 1966).

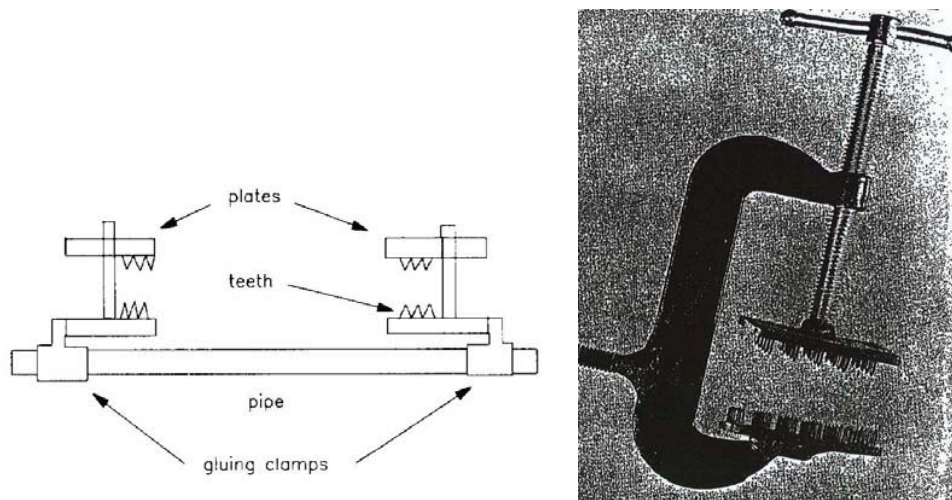


Figure 2.11. Examples of clamping devices used; (a) schematic clamp design used by Bruce & Ball, (1990), (b) enlarged view of one clamp end used by Herring et al., (1967).

Early work by Marsh & Leet (1966) examined the effects of shortening in beef *M. sternomandibularis*. Results indicated that when muscles were shortened by 20% there was no effect on shear force, however there was a large increase in shear force when muscles were shortened to 20-40% (Figure 2.12). When muscles shortened from

40-55% muscles decreased in shear force, but from 55-60% shortening the shear force results were similar to that of muscles shortened by 20%.

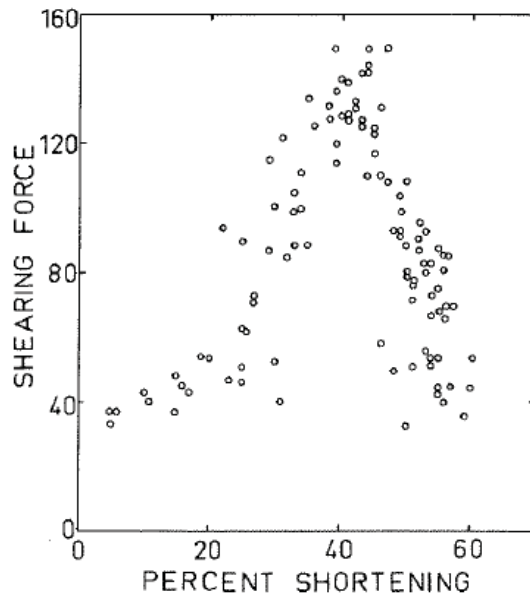


Figure 2.12. Relative tenderness in relation to the shortening induced by transfer of samples from room temperature to 2°C at intervals during *rigor* onset (Marsh & Leet, 1966).

Later Herring et al. (1967) measured the effects on tenderness in beef *M. semitendinosus* when muscles had been shortened by up to 48% or stretched up to 48% (based on pre-excised muscle length). From these results it was concluded that essentially it was more important to prevent *post-mortem* shortening than to ensure a maximum stretch with regard to tenderness. Although there were still differences found when muscles were stretched from 12-48% they were not as evident when compared to the degrees of shortening (Herring et al., 1967). These findings were supported by both Bruce & Ball (1990) and Simmons, Cairney, Auld, Nagle, & Mudford (1999). Bruce & Ball (1990) found lower shear force in beef *semitendinosus* muscles when extended by 25% and chilled at low temperatures. Simmons et al., (1999) stretched beef *longissimus* muscles by either, 20, 40 or 60% and compared the shear force with un-stretched muscles. Significant improvements in shear force were

achieved when muscle was stretched to 20%, but there was no further benefit when muscles were stretched by 40 or 60%.

These mechanical stretching techniques were able to prove the concept that meat that is excised *pre-rigor* can still achieve adequate meat tenderness. This is provided the muscles are prevented from contracting. Based on these studies it also appears that there is an upper threshold where the benefits of stretching are diminished or no further improvement in shear force can be achieved. Despite these benefits there is no real commercial application of this technique given the labour involved to clamp and the potential need for the muscle to remain clamped until the completion of *rigor*. This has led to the development of other methods to restrain and stretch individual muscles.

2.7.2 Muscle wrapping

Devine et al. (1999) developed a technique which tightly wrapped beef *longissimus* muscles using a polyethylene cling film. Since then many studies have examined the impact of this technique on meat quality in both beef and sheep (Devine et al., 2002a; Devine, Payne, & Wells, 2002b; Hildrum, Andersen, Nilsen, & Wahlgren, 2000; Rosenvold, North, Devine, Micklander, Hansen, Dobbie, & Wells, 2008; Toohey, Hopkins, & Lamb, 2008a).

This concept was developed largely to prevent the *pre-rigor* excised muscles from contracting and to therefore mimic the skeletal restraint normally provided by the carcass. Results from Devine et al. (1999) indicated that when *rigor* occurred at higher temperatures 20-35°C the wrapping of beef *longissimus* muscles was effective in reducing muscle shortening and hence improving meat tenderness. However, when muscles entered *rigor* at an optimal temperature of 15°C the sarcomere and meat

tenderness were unaffected. Hence it would seem there would be little value to wrap muscles that enter *rigor* at 15°C.

Additional studies on beef by Hildrum et al. (2000) also reported a significant improvement in tenderness for wrapped hot-boned beef *M. longissimus* after 2 and 9 days of ageing. However Hildrum et al. (2000) in the same study reported on wrapped hot-boned beef *M. semimembranosus* and there was no significant improvement in meat tenderness observed. In a review by Sørheim & Hildrum (2002) the authors proposed that this null effect of wrapping on the *M. semimembranosus* was most likely due to size and shape of the cut and hence the physical difficulty of reducing contraction in the muscle when compared to *longissimus* muscle. Rosenvold et al. (2008) also demonstrated the positive effects of wrapping beef *longissimus* muscle on shear force.

Similar results were found in lambs by Devine et al. (2002a) and Devine et al. (2002b) in that the wrapping technique prevented the muscles from shortening and removed the need for meat to be placed in a temperature controlled environment. The study by Toohey et al. (2008a) examined the impact of wrapping mutton *longissimus* muscle and the results from this study supported the previous work in lamb.

Based on earlier studies conducted by Bouton, Harris, & Shorthose (1972) and Bouton, Fisher, Harris, & Baxter (1973b) it was concluded by Bouton et al. (1973a) that stretched muscles have a greater water holding capacity, which is reported to result in less moisture loss during subsequent ageing. There appear to be contrasting results based on which tests are used as indicators of water holding capacity (drip loss, purge and cooking loss). Toohey et al. (2008a) reported that the wrapped muscles had significantly higher drip loss, but when cooking loss was examined it was unaffected by the wrapping treatment. The latter outcome is supported by cooking loss results

reported by Devine et al. (2002a). Devine et al. (1999) and Rosenvold et al. (2008) both showed that the percentage of drip was unaffected by a wrapping treatment in beef *M. longissimus* and did not report on cooking loss. Based on meat colour results reported on by Devine et al. (2002b) it appears that this wrapping technique has minimal to no impact on objective meat colour traits. This technique is quite labour intensive and the degree to which the meat is restrained is determined by the individual wrapping. To our knowledge this technique is not commercially used to improve meat quality. However this technique is used in both Australia and New Zealand to improve the shape of hot-boned higher value cuts (M. Dorahy, personal communication, February, 10 2010) such as the cube roll HAM 2240 (Anonymous 2005).

2.7.3 Pi-Vac Elasto Pack System®

As a further development of the wrapping technique the Pi-Vac Elasto Pack System® was developed. This system was first reported by Stiebing & Karnitzschky (1996) (cited in Farouk et al., 2009). This system uses elastic film which is placed inside a packaging chamber under vacuum. The highly elastic film is stretched to the inside walls of the chamber. Thereafter the muscle is put into the chamber and pressure is released which causes the elastic film to contract to its original size (Figure 2.13). The elastic film then acts as a barrier inhibiting the shortening of the muscle (Troy, 2006).

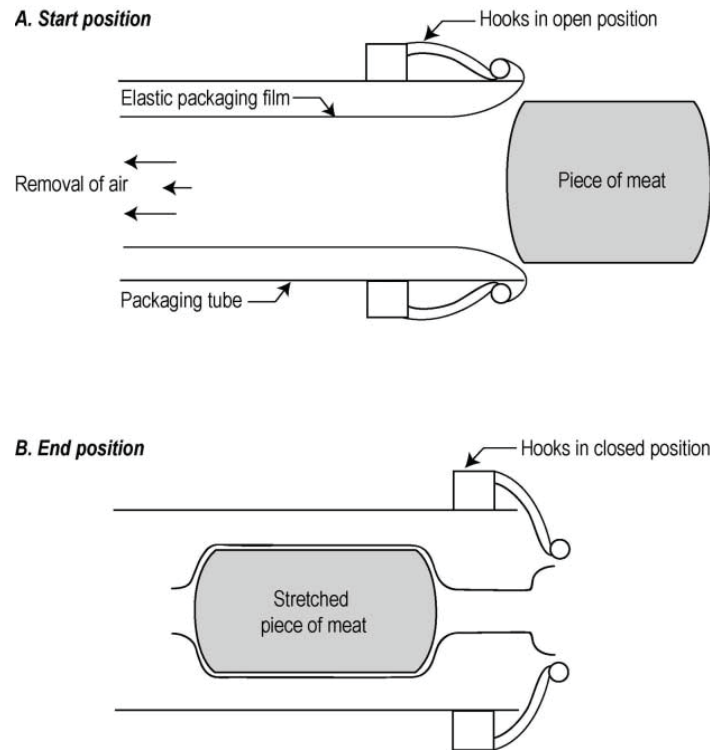


Figure 2.13. Schematic drawings of the Pi-Vac Elasto Pack System® for hot-boned muscles; (A) start position and (B) after filling the machine (Source; Sørheim & Hildrum, 2002).

Initial studies by Wahlgren & Hildrum, (2001) showed an improved tenderness in beef *M. longissimus* when wrapped before *rigor* and held at temperatures of either 4°C or 14°C. This proved that this method had some advantages which included the ability to rapidly chill these muscles without any negative effect on meat tenderness (Wahlgren & Hildrum, 2001) and to achieve a more attractive shape for meat cuts (Hildrum, Nilsen, & Wahlgren, 2002). These results were supported by O’Sullivan et al. (2003). It was shown that by using the Pi-Vac Elasto Pack System® on hot-boned beef (*M. longissimus*) which were excised 90 minutes *post-mortem* sarcomere length and meat tenderness were increased (O’Sullivan et al., 2003). This study also reported that drip loss was significantly reduced and colour was mostly unaffected.

More recently a series of trials that investigated the impact of Pi-Vac Elasto Pack System® on meat quality traits were carried out at The National Food Centre, Dublin,

Ireland and were reported on in a book chapter by Troy, (2006). The first study appeared to present more comprehensive results to those presented by O'Sullivan et al. (2003). This included data on a comparison of Pi-Vac Elasto Pack System® treated and control samples under two chilling treatments 2°C or 10°C which were aged for 7 and 14 days. Similar trends were found irrespective of chilling regime, but the difference between treatments was greater when samples were chilled faster (2°C). As reported by O'Sullivan et al. (2003) the sarcomere length and meat tenderness were increased in Pi-Vac Elasto Pack System® samples and drip loss was decreased.

The second study reported by Troy (2006) examined the comparison of electrical stimulation and Pi-Vac Elasto Pack System® on rapid chilled samples (treatments; Control, high voltage electrical stimulation, low voltage electrical stimulation and Pi-Vac Elasto-Pack System®). Based on the results presented it also appeared that the Pi-Vac Elasto Pack System® had a positive effect on sarcomere length and meat tenderness in the loin, topside and rump although the results were difficult to interpret given not all levels of significance were shown. Despite the benefits reported by both Troy, (2006) and O'Sullivan et al. (2003) these results should be interpreted with caution given that lack of information provided on sample sizes and levels of significance.

Although the Pi-Vac Elasto-Pack System® is a commercial technology designed for hot-boned beef, the industry uptake has been minimal. This in part could be due to the fact that the Pi-Vac Elasto-Pack System® is a non-continuing system which is very labour intensive. It was reported by Sørheim & Hildrum (2002) that further research is being done to improve and incorporate these systems into high-speed production lines however the author is unaware that any new systems have been integrated. Another limiting factor for the Pi-Vac Elasto-Pack System® is that the

foot-print size of the machine is large and it thus requires precious floor space (Figure 2.14). In addition to the authors knowledge this system is not commercially used in the sheep industry.



Figure 2.14. Pi-Vac Elasto Pack System® equipped for four different packaging sizes.

2.7.4. Sarcostretch system

The Sarcostretch system has been described by Farouk et al. (2009). The system is made up of the following parts; (i) three steel loading chambers that taper into three different sized parallel tubes; (ii) a mechanism that shuts and seals the loading chamber after *pre-rigor* muscle is loaded; (iii) a valve to allow air into the chambers to force the muscle down the taper and into a parallel tube; (iv) sensors on the parallel tube to control the movement of the meat through the tube; (v) unstretchable plastic film that meat is pushed into as it exits the parallel tube (Farouk et al., 2009). Once the meat is in the plastic film this then holds the muscle and restricts the muscle from contracting during subsequent *rigor*.

Initial work conducted in 2005 has been reported on in a book chapter by Farouk et al. (2009). It was shown that the Sarcostretch technique stretched *pre-rigor* beef

Mm. semitendinosus, *semimembranosus*, and *biceps femoris* by 97, 78 and 44% respectively. It was concluded that overall tenderness, uniformity, presentation, portion control of meat and drip loss were improved using this method. However it should be noted that no data relating to these meat quality traits was presented hence it is difficult to gauge the effectiveness of Sarcostretch and to the authors knowledge this system has not been commercialised.

2.8 Ageing

Ageing refers to the process where meat becomes tender during the period of refrigerated storage and involves the specific degradation of structural proteins (Hwang et al., 2003a). The knowledge that ageing or conditioning improves meat tenderness is not new and ageing has been a useful tool to improve meat quality (Davey & Gilbert, 1976). The mechanisms by which ageing works to improve meat tenderness involves the breakdown of the structural muscle proteins (e.g. titin, nebulin & desimin) by proteolytic enzymes termed calpains (Devine, 2004). These structural proteins hold the contractile proteins actin and myosin together, however contractile proteins are minimally involved in ageing. Ageing is considered to start after the completion of *rigor*, when the ageing mechanisms are fully activated (Devine, 2004).

The rate and the extent of tenderness improvement during ageing is dependent on both intrinsic factors, such as animal species (Etherington, Taylor, & Dransfield, 1987), muscle type (Stolowski, Baird, Miller, Savell, Sams, Taylor, Sanders, & Smith, 2006), growth rate (Allingham, Harper, & Hunter, 1998), and on extrinsic factors, such as pre-slaughter handling (Devine, Lowe, Wells, Edwards, Edwards, Starbuck, & Speck, 2006), electrical stimulation (Strydom et al., 2005), chilling method (Savell et al., 2005), ageing time (Davey & Gilbert, 1976) and temperature (Dransfield et al., 1992).

A major influence on the rate of ageing is the temperature of the meat and generally at elevated meat temperatures the ageing process is faster than at lower temperatures (Dransfield et al., 1992). As ageing is temperature dependent it is important to understand that some factors that can affect this relationship. For example at high *rigor* temperatures of about 35°C meat may not reach its full ageing potential when compare with *rigor* temperatures of 15°C (Devine, 2004). Davey, Kuttel & Gilbert (1967) first demonstrated that the degree or extent of tenderness improvement during ageing decreases with increased muscle shortening, hence it is critical as previously mentioned (Section 2.2.3) to ensure shortening does not occur.

The practise of storing meat after death to improve meat tenderness has been used for generations and has been reported to have been studied systematically since 1907 (Lawrie & Leward, 2006). It was summarised by Dransfield (1994) that ageing of beef up to three weeks in a chilled environment produces noticeable improvements in tenderness, however it was noted there has been doubt expressed commercially about the operation of such long storage times, the cost involved and the risk of meat spoilage. From Australia's perspective this is not seen as an issue for some export markets as the meat can be suitably aged during freight and shipping times. However for other markets such as frozen or the domestic market this can provide challenges. For example on the domestic market with the drive for fresh produce, animals can be processed one day and then be at the retail level as early as 48 hours after death for sheep and generally 4 to 5 days for beef. Additionally, in more recent times there has also been a drive to not only to reduce costs but also reduce the environmental footprint. Hence to ensure tenderness is not compromised by limiting ageing time other tenderising mechanisms such as stretching should be considered.

2.9 Chilling

The importance and influence of chilling on muscle structure and hence meat quality has already been outlined in section 2.1. From this it was shown that the chilling of carcasses within the first 24 hours *post-mortem*, when muscle is converted to meat, has been shown to be very critical with regards to meat tenderness. It could be summarised that a carcass temperature between approximately 18-25°C at pH 6.0 is required to optimise tenderness (Thompson et al., 2005).

Savell et al. (2005) showed that there are many methods of chilling (e.g. delayed chilling, spray chilling and rapid or blast chilling) which greatly impact the quality and palatability of meat. The effectiveness of chilling method on decrease in muscle temperature and hence meat quality is dependent on factors such as use of electrical stimulation, species type and even within species (depending on the variance in size of carcasses) as these factors will affect the rate of chilling. Although the optimal *rigor* temperature is well established the challenge is to apply this to all muscles across the whole carcass.

As Huff Lonergan et al. (2010) stated in very large carcasses such as beef, and/or sheep carcasses that have a very thick fat cover, there will be a temperature gradient that develops with the outermost muscles experiencing the most rapid cooling and the innermost muscles experiencing the slowest cooling rate. Hence the effect this has on the subsequent tenderness of specific muscles should be considered. This varying effect on different muscles is often a result of fast chilling regimes designed to control of the growth of the pathogenic micro-organisms which is a food-safety concern during the chilling of red-meat carcasses. All processors of meat must comply with the Australian Standard for the Hygienic Production and Transportation of Meat and Meat Products for Human Consumption (AS4696:2007) and ensure that prior to

transportation or within 24 hours of stunning that the surface temperature of carcasses, sides, quarters or bone-in major separated cuts must fall to 7°C or below (Anon, 2007). One advantage of hot boning is that it allows muscle to be chilled more efficiently, however this does increase the risk of muscle contraction. Another concept which is yet to be validated would be to harvest some muscles for example the removal of some of the outer muscles *pre-rigor* in a beef hind leg and then restrain/stretch them to prevent any contraction due to the removal of carcass restraint.

2.10 Conclusions

From the literature it is clear that there are many factors that impact meat quality, however despite the best efforts not all factors can be controlled. It is apparent that *post-mortem* factors have a significant impact on meat quality traits in particular meat tenderness, colour and water holding capacity. With the increasing demand for not only high quality fresh product from consumers, but also the increasing demand for industry to be more energy efficient from a cost and environmental perspective, these *post-mortem* factors are likely to become more critical.

In order for industry to become more efficient by using techniques such as hot boning, from the literature it is clear that additional processing methods in addition to electrical stimulation are required to produce quality product. Early work has shown that either prevention of muscle contraction or stretching muscles is an effective tool in improving meat quality. Over the years various techniques (electrical stimulation, Tenderstretch, Tendercut, mechanical stretching technologies, muscle wrapping, Sarcostretch and the Pi-Vac Elasto Pack System®) have been developed to enhance meat quality however the adoption of some of these technologies has been limited.

2.11 Scope of this thesis

The research in this thesis was designed to investigate whether an alternative processing step with the use of stretching prototype device (licensed as SmartStretch™) could maintain or enhance meat quality of sheep and beef under commercial hot boning situations. The first stage investigated the effect of both stretching and ageing on meat tenderness of hot-boned sheep topsides (*Mm. semimembranosus*, *adductor* and *gracilis*) in combination with electrical stimulation. The intention was to establish whether this additional processing step would further enhance the tenderness of hot-boned sheep topsides irrespective of the stage of *rigor*.

As a follow on, the second stage investigated the effect of both stretching and ageing on meat tenderness, of whole, hot-boned legs from sheep carcasses. The intention was to quantify whether multiple muscles would impact on the effectiveness of the stretching prototype device SmartStretch™ on meat quality of three key muscles (*Mm. semitendinosus*, *semimembranosus* and *biceps femoris*).

The third stage was designed to evaluate whether the use of medium voltage electrical stimulation inhibited the degree of stretch. To test this, the interaction between medium voltage electrical stimulation, stretching and ageing on key meat quality traits including meat tenderness, meat colour and water holding capacity of hot-boned sheep *M. semimembranosus* were examined.

Lastly it was of interest to examine whether the benefits of this stretching prototype could be transferred across species to beef. Two experiments were designed with the first designed to evaluate the effect of both stretch and ageing on meat quality traits of hot-boned beef *M. semimembranosus* taken from cull cows. The second experiment was also designed evaluate the effect of both stretch and ageing on meat quality of hot-boned beef *M. gluteus medius* taken from the Rostbiff of younger cattle.

Chapter 3: Improving the tenderness of sheep topsides (m. semimembranosus) using a meat stretching device

3.1 Introduction

It has been well established that tenderness is one of the most important meat quality traits (for example, Sørheim & Hildrum, 2002; Maltin et al. 2003; Tornberg, 1996). There are three factors that determine meat tenderness which include “background toughness”, the toughening phase and the tenderisation phase (Hopkins & Geesink, 2009). The toughening and tenderisation phases take place during the *post-mortem* storage, in that order, but the impact of the toughening phase can be modified by altering the degree of muscle shortening (Hopkins & Thompson, 2001). This is particularly important in situations such as hot boning where removal of the muscle from the skeleton prior to *rigor* can lead to excessive shortening when meat is exposed to low temperatures (Marsh & Leet, 1966). Thus it is critical that tenderness is not compromised in the quest for accelerated processing and for this reason hot-boning is often performed in conjunction with electrical stimulation. Both Jeremiah, Martin & Murray (1985) and Pisula & Tyburcy, (1996) highlighted the many benefits from hot boning including: reductions in weight loss during chilling and in drip loss during storage, thus higher final yields; reduction in chiller space resulting in energy saving in inputs on refrigeration; faster turnover of meat in the processing plant; reduction in capital costs for buildings; and finally savings in both labour and transport.

Despite the many advantages of hot-boning there are major constraints. Pisula & Tyburcy, (1996) described the limitations of existing plants being able to adopt the technology due to having to make significant changes to their current processing

systems. Another constraint outlined by Spooncer (1993), is the increased risk of bacterial growth. However, Spooncer (1993) also showed that this could be controlled by a combination of drying and cooling. The impact that hot-boning has on meat tenderness is a significant concern with regard to producing quality meat. Hot-boning increases the risk of muscle shortening (Devine et al., 2004) due to the removal of the *pre-rigor* muscle from skeletal constraint and the subsequent shortening which is a major influence on meat tenderness (Törnberg, 1996). Although this shortening can be minimised through the use of electrical stimulation, in order to further improve the tenderness of hot-boned meat another approach to prevent muscle shortening is to restrain the muscles physically until they are in *rigor mortis* (Macfarlane, Harris & Shorthose, 1974).

Early work by Locker (1960); Herring et al. (1967) and Davey et al. (1967) all proved the concept that *pre-rigor* excised muscles could be stretched resulting in improved tenderness. The stretching of major muscles can be achieved by carcass suspension or alternatively by removing the cut from the carcass and clamping using a simple mechanical device. More recently, Devine et al. (2002b) demonstrated that excising and wrapping meat by hand could be used to control sarcomere shortening by stretching the muscle *pre-rigor*. This wrapping technique was then further developed into the Pi-Vac Elasto-Pack System®. Troy (2006) reported on a series of experiments that linked hot-boning of beef carcasses and the use of the Pi-Vac Elasto Pack System®. Broadly, outcomes from these experiments showed that, when compared to the control, the Pi-Vac Elasto Pack System® enabled hot-boned meat to be packaged under restraint and chilled quickly without the risk of cold shortening and hence toughening. It allowed the possibility of extended shelf life and ensured consistent quality.

The adoption of hot or warm boning in the Australian sheep meat processing industry has been limited to the use of adult sheep. This process in most cases has been integrated with electrical stimulation with the intent of not only reducing muscle contraction subsequent to removal from the skeleton, by hastening the onset of *rigor*, but also decreasing the risk of bacterial growth due to high pH (Spooncer, 1993). The only published data on the eating quality of sheep meat processed through this system shows a low level of consumer compliance with only 14% of samples tested achieving the ‘good everyday’ requirement (Toohey & Hopkins, 2006a). In contrast, when adult sheep were processed at the same abattoir, but conventionally chilled and cold boned, product aged for 7 days achieved a high level of consumer compliance with 86% of samples tested achieving a ‘good everyday’ ranking (Hopkins & Toohey, 2006). This ‘good everyday’ score is derived from consumer sensory testing which is explained by Polkinghorne, Watson, Porter, Gee, Scott & Thompson, (1999) and Watson, Gee, Polkinghorne & Porter (2008). In follow up work where hot-boned loin meat was either wrapped and aged for 7 days or unwrapped and frozen at 1 day, the consumer overall liking score was significantly improved from 57 to 64 respectively as was the tenderness score derived from consumer sensory testing from 52 to 64 respectively. This equates to a 14% and 24% improvement in the overall liking and tenderness scores respectively compared to product unwrapped and frozen at 1 day (Toohey et al. 2008a). In addition to these improvements in sensory scores there was a significant reduction in meat toughness based on objective tenderness results with unwrapped frozen meat at 1 day requiring on average 62N of force to be sheared in half compared to 29N for wrapped and 7 day aged meat. In addition to these studies it was also concluded by Rosenvold et al. (2008) that even without electrical stimulation acceptable tenderness levels could still be achieved at high *pre-rigor* temperatures

when a similar wrapping technique as Toohey et al. (2008a) was applied. However there has been no commercial validation using a stretching prototype device (licensed as SmartStretch™).

Hence the aim of this study was to evaluate the effect of both stretching and ageing on meat tenderness of hot boned sheep topsides (*Mm. semimembranosus*, *adductor*, *gracilis*) using a stretching prototype device (licensed as SmartStretch™), under commercial processing conditions where electrical stimulation was applied. The intention was to establish whether this additional processing step would further enhance the tenderness of hot-boned sheep topsides and if tenderness was affected by the stage of *rigor*.

3.2. Materials and Methods

3.2.1 Animals

For testing the effect of the SmartStretch™ prototype machine and *post-mortem* ageing, a total of 40 sheep of mixed sex (ewes and wethers) randomly selected from three different consignments were assessed. The sheep used from these different consignments were of varying backgrounds representing the typical animals processed by the abattoir. However all sheep used were the same breed (Merino) and were all aged between 3 and 4 years old and were classified as mutton (Anonymous 2005).

3.2.2 Experimental Design

The treatment combinations used were; 0 days ageing + SmartStretch™, 0 days ageing + control, 5 days ageing + SmartStretch™ and 5 days ageing + control. These four treatments were randomised to the two samples across carcasses equally but in

such a manner that the four pair-wise treatment comparisons of most interest each were equally replicated within carcasses.

3.2.3 Sample collection

The carcasses were processed under the normal commercial procedures of the abattoir. As a part of this process carcasses were exposed to a number of electrical inputs routinely used by the cooperating abattoir including, a high frequency immobilisation unit, applied for 25-35 secs (2000 Hz, 400 volts, and a maximum current of 9 amps over 7 animals, pulse width of 150 microseconds), moderate frequency immobilisation (800 Hz, 300 peak volts, a constant current of 1.7 amps, pulse width 150 microseconds) applied for 5-7secs, low voltage electronic bleed (15 Hz, 550 peak volts, constant current of 0.8 amps, pulse width 500 microseconds) applied for 20 seconds and post dressing medium voltage electrical stimulation (MVS) with a constant current 1.0 amp and pulse width of 2500 microseconds, but variable frequency across the 6 electrodes (the frequency for electrodes 1 & 2 was set at 25 Hz, 3 & 4 at 15 Hz and 5 & 6 at 10 Hz, with 300 peak volts) applied for 30-35 seconds. Carcasses then passed through a drying room for approximately 35 minutes with an average temperature of 8°C. Following this process both the right and left topsides (*Mm. semimembranosus*, *adductor* and *gracilis*) HAM. 5073 (Anonymous, 2005) from 40 carcasses ($n = 80$) were collected and trimmed removing all fat and placed into their predetermined treatments within 2 hours of death.

3.2.4 Muscle measurements

Some ‘descriptive’ measurements were taken on each of the whole primal topsides including initial length (Li) and initial circumference (Ci) which was measured at two different points. Topsides that underwent the SmartStretch™ treatment were re-measured once the treatment was completed and a final length (Lf) and circumference (Cf) were recorded. From these results both the percentage increase in length and percentage decrease in circumference at sites 1 and 2 were calculated using the following formulas.

$$\text{Length Increase (\%)} = 100 \times (\text{Lf/Li} - 1)$$

$$\text{Circumference decrease (\%)} = 100 \times (\text{Cf/Ci} - 1)$$

3.2.5 Initial pH & Temperature

The pH and temperature were measured in all topsides (in the *M. semimembranosus*) just prior to the application of the stretch treatment. Muscle pH was measured using a glass combination pH probe (potassium chloride) Ionode intermediate junction pH electrode, (TPS Pty Ltd., Brisbane, Queensland) attached to a data recording pH meter (TPS WP-80). While muscle temperature was measured using a stainless steel cylindrical probe attached to the same meter. The pH meter was calibrated before use and at regular intervals using buffers of pH 4 and pH 6.8 at room temperature.

3.2.6 Treatments

The stretch treatment was applied within 2 hours of death. The treatment was achieved using a meat stretching prototype (SmartStretch™), under development by Meat & Livestock Australia and Meat & Wool New Zealand. The SmartStretch™

technology uses a flexible rubber sleeve which is surrounded by four inflatable bladders that are housed within an airtight chamber. To insert meat into the machine air is pumped out of the chamber under vacuum which causes the rubber sleeve to expand. Once meat has been inserted into the chamber the vacuum is removed and air is then pumped into the four inflatable bladders which surround the rubber sleeve causing the meat to be compressed by force perpendicular to the direction of the muscle fibres. This also applies peristaltic action, moving the meat towards the same end of the sleeve that it was inserted into. Air pressure is then applied to the exterior of the sleeve by pumping air into the chamber, forcing the meat upwards and into packaging. The packaging was a 100 µm polyethylene packaging tube which was aimed to constrict the muscle to prevent any subsequent contraction of the muscle. The control samples were vacuum packed and then both control and stretch treatment samples were mixed and packed into cardboard boxes and were frozen after predetermined ageing periods.

The 0 day samples were placed in a freezer within 4 hours of slaughter to replicate the current hot boning process and stored at -22°C until sampling. The 5 day aged samples were chilled at an average temperature of 2°C and following 5 days of ageing the remaining samples were also frozen and stored in a -22°C freezer.

3.2.7 Sample preparation

After a period of frozen storage the topsides were tempered at an average temperature of 21°C for on average 2 hours to allow the *Mm. adductor* and *gracilis* to be dissected from the *M. semimembranosus*. Then sarcomere, shear force, and particle size samples were cut from the *M. semimembranosus* whilst the *M. semimembranosus* was still predominately frozen.

3.2.8 Sarcomere length

Sarcomere length was measured using laser diffraction as described by Bouton, Harris, Ratcliff, & Roberts (1978) on samples aged for 0 and 5 days.

3.2.9 Warner Bratzler shear force

Shear force samples were cut into approximately 65 gram blocks, with dimensions of approximately 60-70mm length, 40-50mm width, 20-25mm thick. These shear force samples were cooked for 35 min in plastic bags at 71°C in a 90 L water bath with a thermoregulator and a 2000 W heating element (Ratek Instruments, Boronia, Victoria, Australia) and measured using a Lloyd Texture analyser as previously described (Hopkins, Toohey, Warner, Kerr, & van de Ven, 2010).

3.2.10 Cooking loss

Samples used for Warner Bratzler shear force determination were weighed pre and post cooking to measure the amount of cooking loss. After cooking the samples were cooled in running water and patted dry using paper towelling prior to weighing. Cooking loss percentage was calculated from the following formula;

$$\text{Cooking loss (\%)} = 100 \times (1 - \text{cooked weight/fresh weight})$$

3.2.11 Particle Size

A 1-2 g sample was collected from the *M. semimembranosus* for 0 day and 5 day aged samples. The particle size analysis method has been described by Karumendu, van den Ven, Kerr, Lamb, Lanza, & Hopkins (2009) and was conducted on 1g samples.

3.2.12 Statistical analysis

Linear mixed model analyses were used to analyse the results for leg weight, initial pH, initial temperature, Warner Bratzler shear force, sarcomere length, cooking loss and particle size to determine the significance of any stretch treatment (control or SmartStretch™) and/or ageing (0 or 5 days) effects. For each trait the model included stretch treatment, ageing and their interaction as fixed effects and included carcass as a random effect with initial pH tested as a covariate where appropriate. The random error variation was allowed to vary across treatment combinations. These analyses were undertaken using ASReml, (Gilmour, Gogel, Cullis, Welham, & Thompson, 2006) via the statistical package *asreml* (Butler, 2009) under R (R Development Core Team, 2009). Fixed effects in ASReml are tested for significance using Wald-related test statistics developed by Kenward & Roger (1997). When tabulated, the denominator degrees of freedom (df) for these Wald tests are rounded down to be conservative.

Additional regression analyses were undertaken to examine relationships between sarcomere length and shear force; sarcomere length and percent increase in length; sarcomere length and initial pH; shear force and percent increase in length; and lastly shear force and initial pH.

3.3. Results

3.3.1 Muscle measurements

The predicted means adjusted for stretch treatment and range for the sheep meat used in this experiment are shown in Table 3.1 to give an indication of the variation within the data. There were no significant differences ($P > 0.05$) across the means for four stretch and ageing treatment combinations for any of the traits shown in Table

3.1, indicating that the random allocation of muscles to treatment groups was balanced. There was significant carcass variability for each trait.

Table 3.1. Predicted mean, standard error (s.e.) and range for leg weight, initial pH and temperature according to SmartStretch™ and control treatments.

Trait	Control		SmartStretch™	
	Mean (s.e.)	Range	Mean (s.e.)	Range
Leg weight (kg)	3.30 (0.07)	2.32-4.20	3.17 (0.07)	2.42-4.10
Initial pH	6.20 (0.03)	5.87-6.63	6.24 (0.03)	5.84-6.72
Initial temperature (°C)	33.9 (0.32)	28.5-37.8	33.0 (0.32)	28.5-37.6

One of the most important aspects that Table 3.1 highlights, is that based on the initial pH and temperature, the *semimembranosus* muscles on average were still in the *pre-rigor* phase with a mean initial pH of 6.22 if pH 6.0 is used as a crude indicator of *rigor*. However, given the range in some values, it suggests that some would be in the stages of *rigor*. The range in the leg weights of the samples processed represents the diversity of the product processed.

There was an average a 24% increase in *semimembranosus* muscle length (11-44%) as a result of the SmartStretch™ treatment and a 24% decrease in circumference (12-42%). SmartStretch™ also transformed the *M. semimembranosus* into a consistent shape (Figure 3.1).



Figure 3.1. Examples of the effect of the SmartStretch™ treatment on the shape of the topside.

Table 3.2. Wald-statistic F-ratios for stretch, ageing, stretch x ageing and covariate initial pH effects on shear force, cooking loss %, sarcomere length and particle size (statistics for stretch and ageing are calculated with stretch x ageing excluded from the model).

Terms	Shear force (N)		Cooking loss (%)		Sarcomere length (µm)		Particle size (µm)	
	Df	F-ratio	Df	F-ratio	df	F-ratio	df	F-ratio
Stretch	^	^	1,73	35.3***	1,47	134.3***	1,75	3.71 ^{ns}
Age	^	^	1,73	1.10 ^{ns}	1,25	4.02 ^{ns}	1,75	8.90*
Age x Stretch	1,37	10.74**	1,38	0.05 ^{ns}	1,39	0.02 ^{ns}	1,37	5.60 ^{ns}
Initial pH	1,56	1.45 ^{ns}	#	#	1,40	0.52 ^{ns}	#	#

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$, ^{ns} $P > 0.05$, ^ Not tested because there was a significant interaction, # was not used as covariate in model.

3.2 Warner Bratzler shear force

Table 3.2 shows the significant treatment effects and relevant covariates on shear force, cooking loss, sarcomere length and particle size. For shear force the interaction between stretch and ageing treatment was significant ($P < 0.01$). This interaction showed that the 0 day aged control group (no SmartStretch™) meat was the toughest and 5 day aged SmartStretch™ treatment was the most tender (Table 3.3). The carcass effect (fitted as random) was significant indicating that some carcasses have muscle that may not respond as well to the SmartStretch™ treatment.

Table 3.3. Predicted means and standard error (s.e.) of *M. semimembranosus* shear force in newtons, according to treatment groups.

Treatments	Shear force (N)	
	SmartStretch™	Control
0 days aged	39.6 (1.0) b	74.9 (2.2) d
5 days aged	33.8 (1.0) a	53.5 (2.2) c

Means followed by a different letter are significantly different ($P < 0.001$).

The present study also examined the impact of treatments on the distribution of shear force. Figure 3.2, shows that the variation in shear force of the SmartStretch™ treatment groups after both 0 and 5 days of ageing was less than the control treatment groups. The error variance differed significantly ($P < 0.05$) for the SmartStretch™ samples (9.6 ± 4.2) and the control samples (87.7 ± 21.8).

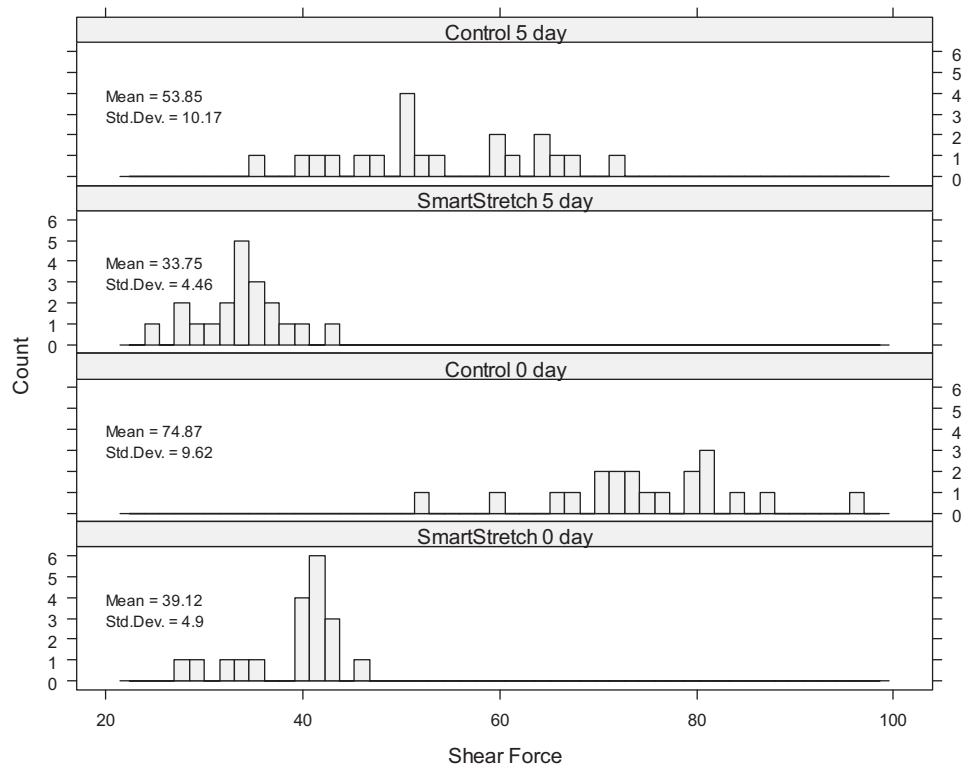


Figure 3.2. Histogram showing the effect that the SmartStretch™ and control treatment at 0 and 5 days had on shear force (N) distribution.

3.3.3 Cooking loss

Cooking loss results reveal a significant difference between SmartStretch™ treatment and control ($P < 0.001$) as shown in Table 3.2 such that SmartStretch™ had significantly lower cooking loss with means (\pm s.e.) equal to 20.2 ± 0.46 and 23.9 ± 0.46 respectively. This trait was not significantly affected ($P > 0.05$) by ageing period and there was no significant interaction between the SmartStretch™ and ageing treatments (Table 3.2).

3.3.4 Sarcomere Length

There was a significant difference ($P < 0.001$, Table 3.2) between SmartStretch™ treatment and control for sarcomere length with means of $2.20 (\pm 0.05) \mu\text{m}$ and 1.57

(± 0.02) μm respectively with an s.e.d. equal to 0.054 μm . There was no significant difference ($P > 0.05$) between ageing periods or interaction between ageing and SmartStretch™ treatment (Table 3.2).

The impact of stretch treatment on the variation or distribution of sarcomere length was examined (Figure 3.3) and the results showed that there was greater variation in the SmartStretch™ treatment for sarcomere length.

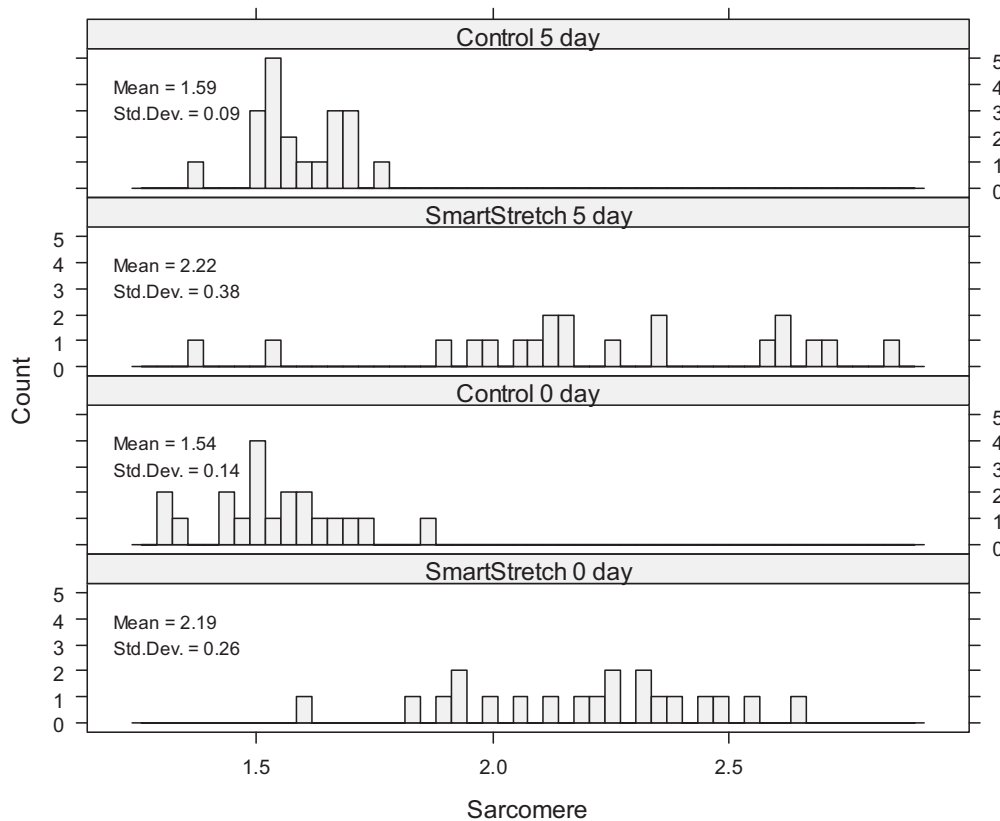


Figure 3.3. Histogram showing the effect of SmartStretch™ and control treatment at 0 and 5 days on the distribution of sarcomere length (μm).

3.3.5 Particle Size

Particle size (PS) results showed that there was no significant difference ($P > 0.05$) between SmartStretch™ treatment and control or interaction between ageing and SmartStretch™ treatment, but there was a significant difference ($P < 0.001$)

between ageing periods (Table 2). As such 5 day aged samples had a lower PS than 0 day aged samples at $157 (\pm 6.7) \mu\text{m}$ and $185 (\pm 6.7) \mu\text{m}$ respectively with an s.e.d. of $9.27 \mu\text{m}$.

3.3.6 Regression analysis

To determine whether the stage of *rigor* impacted on shear force initial pH was included initially as a linear effect, however it was determined not to be significant (Table 3.2; $P = 0.23$). In addition to this there was no significant relationship ($P = 0.80$) shown between initial pH and sarcomere length (Table 3.2). If the shear force and sarcomere length results for samples within each treatment were examined separately, there was no significant correlation (at the 0.05 level) for any treatment between these two traits (Figure 3.4). However there was a marginally significant ($P \approx 0.10$) negative correlation with control samples aged for 5 days.

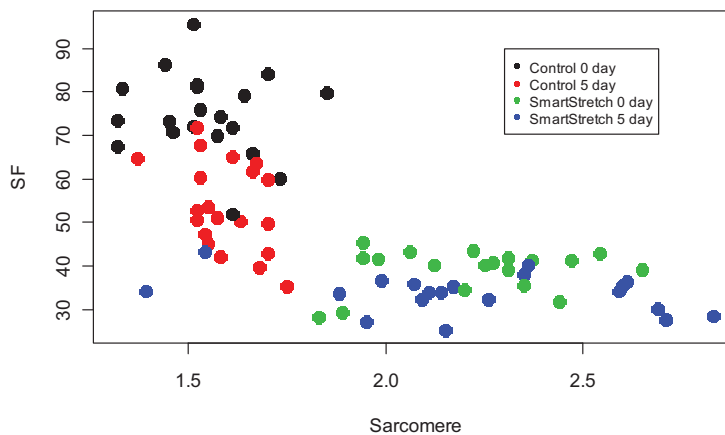


Figure 3.4. Regression analyses of shear force (SF) measured in Newtons and sarcomere length (μm) for each stretch and ageing treatment.

Figure 3.4 does indicate, ignoring treatment, a clear relationship between the two traits (shear force and sarcomere length). Regressing $\log(\text{Shear force})$ on sarcomere

length ignoring treatments using a spline model gave a significant trend (Figure 3.5) suggesting a threshold in sarcomere length at about 2.0 μm , above which shear force plateaus.

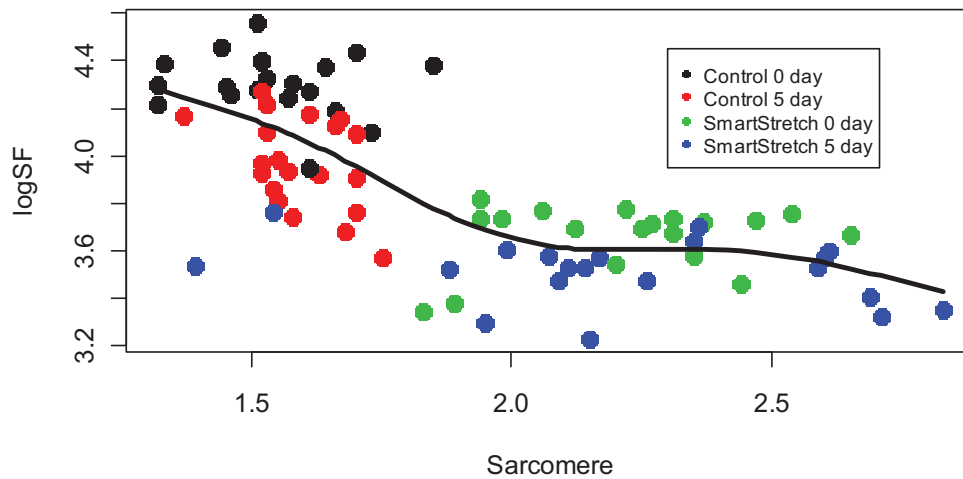


Figure 3.5. Spline analysis for logSF (Newtons) and sarcomere length (μm).

When the residuals about the trend were examined, separately for each treatment, via box-plots (Figure 3.6), the residuals are not independent and identically distributed $N(0, \sigma^2)$. This indicates that logSF is not solely related to sarcomere length and that the treatments are having some additional effect causing differences in shear force.

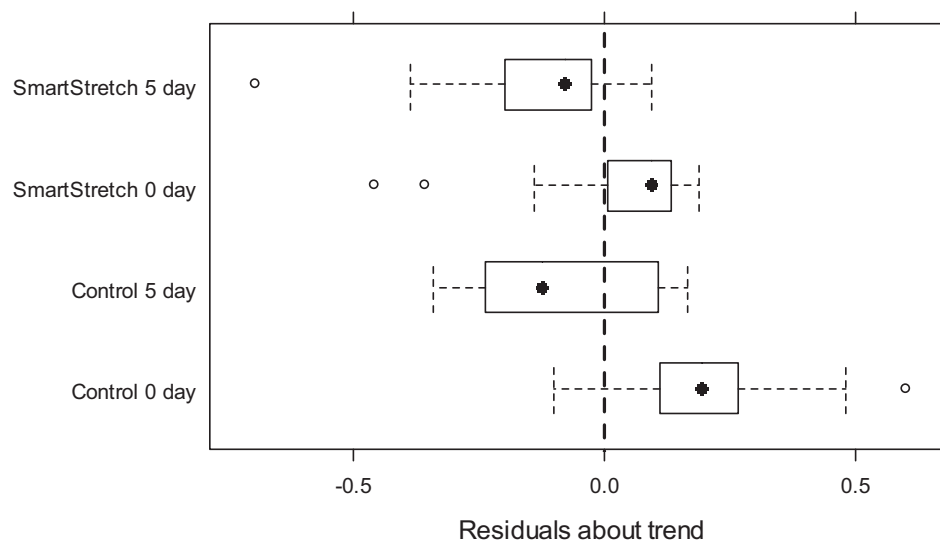


Figure 3.6. Box-plot of the residuals about the trend for each stretch and ageing treatment.

The percent increase in muscle length did not show a significant relationship with sarcomere length ($P = 0.55$) or shear force ($P = 0.42$).

3.4 Discussion

In the current study 0 day aged SmartStretch™ treatment caused a reduction of 46% or 34N in shear force relative to muscle that was allowed to shorten during *rigor*. Even after 5 days of aging there were still large benefits evident, with a reduction in shear force of 38% or 20N. It can be concluded that without the use of SmartStretch™ the product was extremely tough at day 0 and was still far from acceptable at day 5. These improvements in shear force caused by the SmartStretch™ are comparable to those reported by Hopkins et al. (2000b) by using Tenderstretching and super Tenderstretching of the loin where on average tenderness was improved by 40%. This dramatic improvement was also observed in earlier work by Bouton et al., (1973a), which examined the impact of Tenderstretch on sheep *M. semimembranosus* amongst other muscles. In a more recent study by Thompson et al. (2005) in both lamb and

sheep meat it was shown that although shear force differences were not as dramatic, Tenderstretching also resulted in an improvement in eating quality. In this study by Thompson et al. (2005) the effect appeared to be a function of *rigor* temperature meaning if muscles entered *rigor* at the optimal temperature there wasn't much difference, but if heat or cold shortening occurred the difference was greater.

The mechanisms by which SmartStretch™ impacts on meat quality has many similarities to the principles of Tenderstretching as both techniques are applied on *pre-rigor* muscle. Tenderstretching is achieved by suspending carcasses from the aitch bone (*obturator foramen*) in beef or the pelvis in sheep as it comes off the slaughter chain, hence placing increased tension on major leg muscles (*Mm. semimembranosus, gluteus medius, vastus lateralis, biceps femoris*) and loin (*M. longissimus*) before they pass through *rigor* (Thompson et al., 2005; Hopkins, 2004). As reported by Thompson (2002) the increased tension is aimed at either, stretching or minimising shortening (reducing the overlap of actin and myosin) of muscles and hence improving meat tenderness. However it has been reported that super Tenderstretching resulted in clear breaks in the I-band and disassembly of the Z disks indicating disruption of proteins such as actin, titin and nebulin, thus weakening the sarcomere and increasing tenderness, but it is unlikely that SmartStretch™ can create this level of disruption.

Although there are parallels between the two techniques of SmartStretch™ and Tenderstretch there are many differences. For example, SmartStretch™ is performed on excised hot-boned primals and Tenderstretch is performed on whole carcasses and as a result it would be expected that commercially there would be differences in cooling rates. These differences are important to remember when the comparing

techniques. The study of Locker & Hagyard, (1963) and the more recent work by Devine et al. (2002b) stressed the impact of carcass restraint and *rigor* temperature with regard to meat tenderness.

As expected ageing provided improvements in shear force and in addition to these ageing benefits there was also a significant interaction between stretch and ageing treatments. The interaction shown in the current study supports the outcomes reported by Devine et al. (1999) in that hand wrapped hot-boned beef *M. longissimus* had significantly lower shear force values when compared to control *M. longissimus* after 0 and 7 days of ageing. In addition to this Hildrum et al. (2000) also reported a significant improvement in tenderness for wrapped hot-boned beef *M. longissimus* after 2 and 9 days of ageing. However, Hildrum et al. (2000) in the same study reported on wrapped hot-boned beef *M. semimembranosus* and there was no significant improvement in meat tenderness. It was concluded by Sørheim & Hildrum (2002) that the null effect of wrapping reported by Hildrum et al. (2000) for the *M. semimembranosus* was most likely due to size and shape of the cut and thus the physical difficulty of reducing contraction in the muscle when compared to *M. longissimus* muscle. In addition to these studies Rosenvold et al. (2008) and Troy (2006) also reported on the benefits of constricting *pre-rigor* muscle contraction through wrapping and the use of Pi-Vac Elasto Pack System® respectively on shear force.

Given that the primary cause of consumers not repurchasing red meat is due to the variability in eating quality, especially meat tenderness (Bindon & Jones, 2001) the present study examined the impact of treatments on the distribution of shear force. It was shown that the variation in shear force of the SmartStretch™ treatment groups after both 0 and 5 days of ageing is less than the control treatment groups. This

supports outcomes reported by Devine et al. (1999) which showed that by wrapping meat smaller variation in shear force was achieved compared to unwrapped meat. This indicates that by restricting the muscle from contracting one can reduce the variation in meat tenderness, thus producing a more consistent product.

Based on earlier studies conducted by Bouton et al. (1972) and Bouton et al. (1973b) it was concluded by Bouton et al. (1973a) that stretched muscles have a greater water holding capacity, which is reported to result in less moisture loss during subsequent ageing. However, there appears to be contrasting results, as both, O'Sullivan et al. (2003) and Troy (2006) reported a reduced drip loss as a result of Pi-Vac Elasto Pack System® treatment of hot boned beef *M. longissimus*. This was not observed in the study by Toohey et al. (2008a) where sheep *M. longissimus* was wrapped using the same method as Devine et al. (1999). In contrast, it was found that the wrapped treatment had significantly higher drip loss, but when cooking loss was examined it was unaffected by the wrapped treatment. Devine et al. (1999) and Rosenvold et al. (2008) both showed that the percentage of drip was unaffected by a wrapping treatment when *M. longissimus* from hot-boned beef was examined. When Devine et al. (2002b) examined cooking loss in hot-boned sheep *M. longissimus* there was no significant difference between wrapping and control treatments or ageing treatments, supporting the results of Toohey et al. (2008a). Although in the current study there was a significant difference between the SmartStretch™ treatment and control for cooking loss at 0 and 5 days ageing, this result may be attributed to the varying amount of initial purge that was lost before samples were processed. Given that purge has not been reported on in any of the above studies, the author of the current study concludes that further investigation into the impact that wrapping,

constricting or stretching the muscle has on overall water holding capacity of individual muscles needs to be conducted.

Excised-restrained muscle strips from the *M. semimembranosus* had longer sarcomeres compared to control and there was no significant change in sarcomere length from 1 to 3 days *post-mortem* (Suzuki, Yamadera, Kido, & Watanabe 1997) supporting the findings in the current study. Similar outcomes were also shown by Devine et al. (2002b) and Devine et al. (1999) where sarcomere shortening was controlled by wrapping meat. This wrapping method is a similar concept to the SmartStretch™ as the aim was to restrain and hence potentially stretch the de-boned muscle to prevent the muscle fibres contracting or shortening. In addition, results from studies using the Pi-Vac Elasto Pack System® (again using the same principles of preventing muscle fibres contracting by placing an overwrap) found sarcomere lengths to be significantly longer when compared to the control group (Hildrum et al., 2000; O'Sullivan et al., 2003; Troy 2006).

The impact of stretch treatment on the variation or distribution of sarcomere length indicated that there was greater variation in the SmartStretch™ treatment for sarcomere length. This is an interesting outcome given muscle shortening is known as a cause of meat toughness (Macfarlane et al., 1974) and in the current experiment SmartStretch™ reduced the variation in shear force. However, based on previous studies the relationship between shear and sarcomere length would appear to be complex and varied. Many studies have examined the relationship between sarcomere length and shear force (e.g. Smulders, Marsh, Swartz, Russell, & Hoenecke, 1990) and conclusions on this relationship have been far from unanimous. Multiple studies have found the relationship to be strong such that as shear force increases sarcomere values decrease (Locker & Hagyard, 1963; Herring et al. 1965; Herring et al., 1967;

Bouton et al., 1973a; Davis, Smith, Carpenter, Dutson, & Cross., 1979; Devine et al., 1999) and other studies have found no relationship (Culler, Parrish, Smith, & Cross, 1978; Parrish, Vandell, & Culler, 1979; Smith, Kastner, Hunt, Kropf & Allen, 1979; Seideman, Koohmaraie, & Crouse, 1987; Shackelford, Koohmaraie & Savell, 1994; Koohmaraie, Shackelford, Wheeler, Lonergan, & Doumit, 1995). Therefore why are there such contrasting results? Previous studies show that a number of factors may impact on the relationship between shear force and sarcomere. These factors include: rate of glycolysis (Smulders et al., 1990) and thus the temperature at *rigor mortis* (Devine et al., 1999; Devine et al. 2002a; Geesink et al. 2001) and thresholds for sarcomere length (Marsh, & Leet, 1966; Macfarlane et al., 1974). Based on conclusions from these previous studies it is not surprising that there is not a simple linear relationship between shear force and sarcomere length given the range in both initial pH (5.84-6.72) and sarcomere length (1.32-2.83 μ m) data. As shown with the regression analysis (spline model) for log (shear force) on sarcomere length, shear force tends to remain constant when sarcomere length is greater than about 2 μ m.

The stage of *rigor* can impact the effectiveness of the stretch treatment, where the onset of *rigor* can be defined crudely as when the muscle reaches a pH of 6.0 (Hwang et al., 2003a). However it is known that this does not occur across all muscle fibre simultaneously (Jeacocke, 1984) and is dependent on initial glycogen levels (Hwang et al., 2003a). If muscles have reached *rigor mortis* meaning adenosine triphosphate (ATP) is depleted, and permanent cross bridge formation of the contractile components actin and myosin occurs (Hwang et al., 2003a) the muscle becomes stiff and the stretch treatment would be less effective. Based on the initial pH and temperature of the *M. semimembranosus* in the current study, muscles were on average still in the *pre-rigor* phase with a mean pH of 6.22 ± 0.18 and a mean

temperature of 33.5 ± 2.02 . This indicates that some muscles would have entered the stages of *rigor*. However it does not appear that this has significantly impacted the effectiveness of the stretch treatment given the relationship between initial pH and both shear force and sarcomere length was not significant. Hence the initial pH does not explain any of the variation in shear force or sarcomere length. In addition the present study also investigated the relationships between the percent increase in length of the muscle for both sarcomere length and shear force. It was found that there was no relationship between either traits and therefore the percent increase in length also has not explained any of the variation in shear force or sarcomere length. This indicates that there are other factors driving the changed in shear force.

Particle size analysis is one approach to measuring the degree of proteolysis that has occurred in meat and has been found to explain some of the variation in shear force (Karumendu et al., 2009). Results from the current study showed that the stretch treatment had no effect on proteolysis, but the ageing treatment did. As such 5 day aged samples had a lower particle size than 0 day aged samples, indicating that the fibres had degraded more during ageing, an outcome supported by the results of Karumendu et al. (2009). This degradation was not influenced by the stretching treatment and is, as expected, due to ageing (e.g. Koohmaraie, Doumit, & Wheeler, 1996). Given stretching treatment did not impact the rate of protein degradation, the improvement in tenderness must reflect a change in sarcomere structure independent of proteolysis, an aspect that is worthy of further investigation.

The SmartStretch™ treatment caused on average a 24% increase in *semimembranosus* muscle length and a 24% decrease in circumference, hence transforming the *M. semimembranosus* into a consistent shape. This consistency is considered a desirable trait by the food service industry (Anonymous, 2003) as it

standardises shape for the preparation of roasts and slices and minimises cutting losses. In addition to this Tarrant (1998) reported that consumer demand has also shifted to more uniform, small pre-packaged retail portions. This is an important industry outcome as to our knowledge there is no other machine that can take a whole primal (*M. semimembranosus*) and shape it to a consistent size and shape.

3.5 Conclusion

Meat tenderness of the *M. semimembranosus* was improved significantly by applying the SmartStretch™ treatment and the benefits were still evident after 5 days of ageing. There was no evidence based on measurement of particle size that the SmartStretch™ treatment impacted on protein degradation, so the improvements from this treatment appear to partially reflect an increase in sarcomere length. It was also shown that the SmartStretch™ treatment resulted in less cooking loss. Further work needs to be completed to determine the impact SmartStretch™ has on other indicators of water holding capacity traits such as purge. This study has highlighted the potential to the improve sheep meat quality and consumer satisfaction for a lower grade hot-boned product using SmartStretch™. It remains to be seen whether this benefit can be translated to other primals in the sheep carcass.

Chapter 4: Improving the tenderness of leg meat from sheep using a meat stretching device

4.1 Introduction

Given the potential negative effect that hot boning can have on eating quality, the adoption of hot boning in the Australian sheep meat processing industry has been limited to adult sheep carcasses. Chapter 3 highlighted there are many advantages and disadvantages with hot boning (Pisula & Tyburcy, 1996; Spooncer, 1993; Jeremiah, Martin, & Murray, 1985 and Devine et al., 2004). However it was also noted that with additional processing interventions such as electrical stimulation and some form of stretching intervention the overall meat quality, in particular tenderness, can be improved (Macfarlane, Harris, & Shorthose, 1974; Locker 1960; Herring et al. 1967; Davey et al. 1967; Devine et al. 2002b; Troy 2006).

The effect of both, stretching and ageing on the tenderness of hot boned sheep topsides (*M. semimembranosus*) using a pre-production stretching prototype device called SmartStretch™ was outlined in Chapter 3. This work showed that tenderness of the *M. semimembranosus* was significantly improved by applying the SmartStretch™ treatment, such that after 0 days of ageing the SmartStretch™ treatment caused a 46% (or 34N) reduction in shear force and a 38% (or 20N) reduction after 5 days of ageing compared to non-stretched meat. Based on these results it was concluded that the accelerated tenderness achieved by this *pre-rigor* stretching device could remove the need for aged chiller storage of hot-boned product to achieve acceptable tenderness levels.

This chapter describes the results of an examination of the effect of stretching, using a prototype device (SmartStretch™), and ageing on meat tenderness, of whole,

hot-boned legs from sheep carcasses. The intention was to quantify whether this additional processing step (SmartStretch™) would further enhance the tenderness of hot tunnel boned sheep legs as it did with the *M. semimembranosus* as outlined in Chapter 3.

4.2 Materials and Methods

4.2.1 Animals

For testing the effect of the SmartStretch™ prototype machine and *post-mortem* ageing, a total of 40 sheep from various consignments were assessed over two kill days. The sheep used were randomly selected from different consignments and thus were of varying backgrounds, representing the typical animals processed by the abattoir. The sheep were mixed sex (ewes and wethers), Merino and were estimated to be aged between 3 and 5 years old.

4.2.2 Experimental design

Using a randomised complete block design with 10 replicates, the treatments were randomised to hindleg within a carcass. The treatment combinations were; ageing 0 days + SmartStretch™, ageing 0 days + control, ageing 5 days + SmartStretch™, ageing 5 days + control. The treatment combinations were randomised between carcass and side within carcass.

4.2.3 Sample collection

The carcasses were processed under the normal commercial procedures of the abattoir. As a part of this process, carcasses were exposed to a number of electrical inputs routinely used by the cooperating abattoir as previously described in section 3.2.3. Carcasses then passed through a drying room for approximately 35 minutes at an average temperature of 8°C. Following this process both the left and right legs (Anonymous 2005; HAM. 5060) were excised from the carcasses *pre-rigor* using a tunnel boning technique ($n = 80$). Then the boneless legs were trimmed to approximately < 10mm of subcutaneous fat and subjected to their predetermined treatments.

4.2.4 Muscle measurements

Some descriptive measurements were taken on each of the legs including initial length and initial circumference which were measured at two different points. Legs that underwent the SmartStretch™ treatment were re-measured once the treatment was completed. From these values a percentage change was calculated, as previously outlined in section 3.2.4.

4.2.5 Initial pH & Temperature

The initial pH and temperature were measured in both the left and right *Mm. semimembranosus* and *semitendinosus* of each sample as soon as the legs were collected and just prior to the application of the stretch treatment. These measurements were taken using a TPS WP-80 pH meter as previously described in section 3.2.5.

4.2.6 Treatments

The stretch treatment was applied within 2 hours of exsanguination. The treatment was achieved using a meat stretching prototype (SmartStretch™) as previously described in section 3.2.6. The 0 day samples were placed in freezer within 4 hours of slaughter to replicate the current hot boning process and stored at -22°C until sampling. The 5 day aged samples were chilled at an average temperature of 2°C and then also frozen and stored in a -22°C freezer.

4.2.7. Histology

A 1 g sample was taken from the lateral side of the *M. semimembranosus* aged for 0 and 5 days. The 0 day aged sample was collected within 2 hours of exsanguination and then samples were collected after 5 days of ageing. The muscle was fixed in a solution of 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1M phosphate buffer and was subsequently used to determine the number of breaks in muscle fibres. The method for determining fibre breaks was adapted from that reported by Taylor & Frylinck (2003). This involved the fixing, embedding and staining of muscle samples. Digital images were collected at 40x magnification using a Leica DMR microscope and Nikon DXM1200F digital camera. Breaks across the fibres were quantified for 40 fibres per sample and if the fibres were not flat, but distorted, this was also recorded.

4.2.8 Sample preparation

The 0 day aged frozen whole boneless legs were tempered for approximately 6 hours at an average room temperature of 21.4°C to allow the *Mm. semimembranosus*, *semitendinosus* and *biceps femoris* to be excised.

The 5 day aged whole boneless legs were thawed to a muscle temperature of approximately 2°C at a room temperature of 21.7°C. This enabled purge to be measured on the whole leg and the *Mm. semimembranosus*, *semitendinosus* and *biceps femoris* to be separated.

4.2.9 Final pH

A 1 g sample was taken from the *Mm. semimembranosus*, *semitendinosus* and *biceps femoris* for determination of final pH on 0 and 5 day aged samples. This was determined using an iodoacetate method adapted from that described by Dransfield, Etherington, & Taylor (1992). The 1 g sample was added to 6ml of cold iodoacetate (at pH 7) and homogenised at 19,000 rpm for two bursts of 15 seconds. Samples were held on ice for 30 seconds between each burst. Following this the samples were incubated in a water bath at 20°C and the pH was measured.

4.2.10 Purge loss

Purge was measured in 5 day aged leg samples only. The initial frozen leg weight was first recorded then the legs were thawed at a room temperature of 21.7°C. Once samples had thawed (muscle temperature of approximately 2°C) they were patted dry using paper towelling and re-weighed to get a final leg weight. The total purge percentage was calculated using the following formula;

$$\text{Purge loss (\%)} = 100 - (\text{Final leg weight/Initial leg weight} * 100)$$

4.2.11 Sarcomere length, Warner Bratzler shear force, cooking loss and particle size

These measurements were taken and conducted as described in sections 3.2.8, 3.2.9, 3.2.10 and 3.2.11 respectively. Sarcomere length was measured on the *Mm. semimembranosus* and *semitendinosus* in 0 day aged samples.

Shear force samples were collected from both the *Mm. semimembranosus* and *biceps femoris* after 0 and 5 days of ageing. Cooking loss was measured on these samples. Particle size samples were collected from the *M. semimembranosus* aged for both 0 and 5 days.

4.2.12 Statistical analysis

The statistical analysis was undertaken using the same methods as described in section 3.2.12. The model contained fixed effects for SmartStretch™ treatment (control or SmartStretch™), ageing time (0 or 5 days) and the interaction. The random terms used in the model were consignment, replicate and carcass. There were 10 replicates derived from the four treatment combinations within carcass.

Regression analysis was used to derive the relationship between sarcomere length and shear force, sarcomere length and percent increase in length, sarcomere length and initial pH, shear force and percent increase in length and lastly shear force and initial pH. The model fitted for the linear mixed model regression analysis was $Y = \text{baseline} + X + \mathbf{Carcass} + \mathbf{error}$ where the random terms are in bold and $Y =$ (shear force or sarcomere length) and $X =$ (sarcomere length, percent increase in length or initial pH). Note that percent increase in length was only available for stretched samples and sarcomere length was only available for 0 day aged samples.

4.3 Results

4.3.1 Muscle measurements

The predicted mean, standard error and range for the various carcass characteristics and meat quality indicators are shown in Table 4.1. There was no significant difference ($P > 0.05$) between stretch and ageing treatments for any of the traits shown in Table 4.1 nor were there any interactions. This indicates that the random allocation of muscles across treatment groups was balanced. There was however for final *M. semimembranosus* pH a significant interaction between stretch and ageing treatments ($P = 0.02$) such that 0 day control treatment had a significantly lower pH (5.77) compared to 5 day control (5.87).

Table 4.1. Predicted mean, standard error (s.e.) and range of leg weight, initial pH and temperature for *semimembranosus* (SM), *semitendinosus* (ST) and final pH for *semimembranosus* (SM), *semitendinosus* (ST) and *biceps femoris* (BF) according to SmartStretch™ and control treatments.

Trait	Control		SmartStretch™	
	Mean (s.e.)	Range	Mean (s.e.)	Range
Leg weight (kg)	1.96 (0.05)	1.37-2.68	1.92 (0.05)	1.40-2.47
Initial pH – SM	6.15 (0.03)	5.91-6.57	6.17 (0.03)	5.77-6.65
Initial temperature (°C) – SM	27.8 (0.41)	20.9-32.1	27.5 (0.42)	21.8-33.4
Final pH – SM	5.79 (0.05)	5.44-6.73	5.85 (0.05)	5.52-6.95
Initial pH – ST	6.30 (0.04)	5.68-6.92	6.30 (0.04)	5.91-6.96
Initial temperature (°C) – ST	24.7 (0.40)	18.2-30.4	24.6 (0.40)	20.1-30.2
Final pH – ST	6.24 (0.05)	5.59-7.00	6.27 (0.05)	5.65-6.94
Final pH – BF	5.97 (0.05)	5.54-6.73	6.03 (0.05)	5.48-6.77

Based on the initial pH and temperature, the *Mm. semimembranosus* and *semitendinosus*, on average were still in the *pre-rigor* phase (Table 4.1) however given the range in initial pH for both muscles, it suggests that some carcasses would be in stages of *rigor*. The range in the leg weights of the samples processed represents the diverse product processed. This was also evident, based on the random terms fitted

in the model (consignment, replicate and carcass) where the carcass variance component was significant for all traits shown in Table 4.1.

On average a 14% increase in whole leg length occurred as a result of the SmartStretch™ treatment with a range in raw data from 2-24%. After the SmartStretch™ treatment was applied a average 44% decrease in circumference occurred with a range in raw data from 21-65%.

4.3.2 Warner Bratzler shear force

The significance of treatment effects and relevant covariates on shear force for both the *semimembranosus* and *biceps femoris* muscles is shown in Table 4.2. There was a significant interaction between stretching and ageing treatments ($P < 0.05$) for the *M. semimembranosus* shear force, but none of the random terms fitted in the model were significant.

Table 4.2. Wald-statistic *F*-ratio for effects stretch, age, stretch x age and covariate initial pH on shear force of *Mm. semimembranosus* (SM) and *biceps femoris* (BF).

Terms	Shear force SM (N)		Shear force BF (N)	
	df	<i>F</i> -ratio	df	<i>F</i> -ratio
Stretch	^	^	1,52	9.51**
Age	^	^	1,50	39.18***
Age x Stretch	1,35	4.16*	1,36	2.32 ^{ns}
Initial pH	1,58	2.34 ^{ns}	1,58	2.34 ^{ns}

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$, ^{ns} $P > 0.05$, ^ Not tested because there is a significant interaction.

The predicted means for the *M. semimembranosus* shear force are shown in Table 4.3. The interaction for the *M. semimembranosus* showed that the un-aged control group (no SmartStretch™) meat was the toughest. The benefits of the stretching treatment diminished after 5 days of ageing.

Table 4.3. Predicted means (s.e.) for shear force of the *M. semimembranosus* (SM) according to treatment groups.

Treatments	Shear force SM (N)	
	SmartStretch™	Control
0 days aged	51.3 (2.87) b	60.5 (3.40) c
5 days aged	40.6 (2.34) a	38.8 (2.12) a

Means followed by a different letter within rows and columns for traits are significantly different ($P < 0.05$).

For the *M. biceps femoris* shear force both stretch ($P < 0.01$) and ageing ($P < 0.001$) were significant, but there was no significant interaction ($P > 0.05$) between the two treatments (Table 4.2) and none of the random terms fitted in the model were significant. The SmartStretch™ treatment significantly improved meat tenderness when compared to control with means of 36.3 (se \pm 1.57) and 41.8 (se \pm 1.57) newtons respectively. As ageing increased the meat tenderness improved in the *M. biceps femoris* with the mean 0 day shear force being 44.8 (se \pm 1.55) and the 5 day shear force 33.3 (se \pm 1.59) newtons.

The present study also examined the impact of the treatments on the distribution of shear force for both the *Mm. semimembranosus* and *biceps femoris* as shown in Figures 4.1-4.2 respectively. These results indicate that the degree of variation for stretch treatment is inconsistent between muscles and days aged in *M. semimembranosus*.

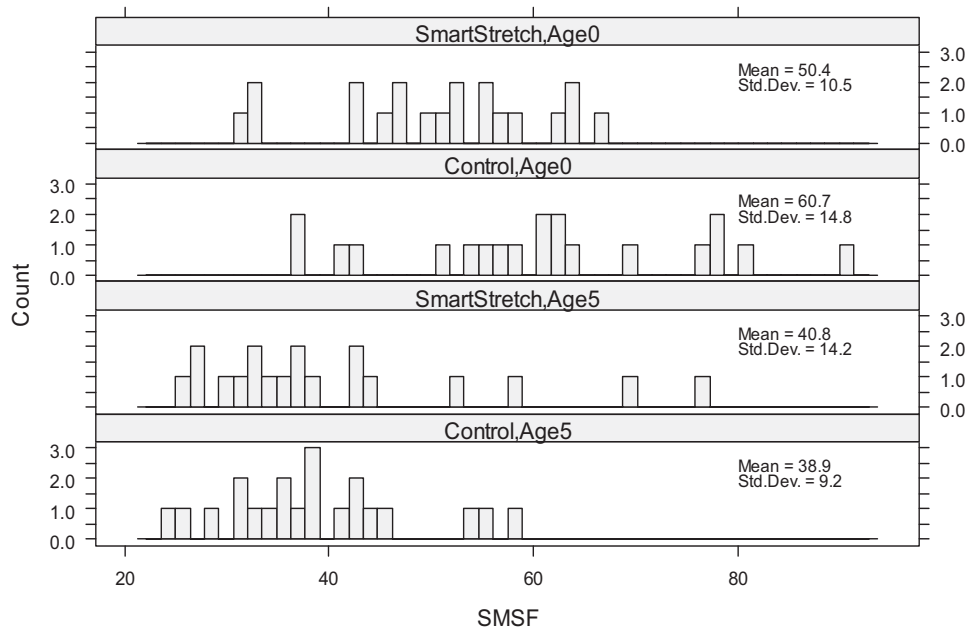


Figure 4.1. Histogram showing the effect of SmartStretch™ and control treatment at 0 and 5 days on shear force (N) distribution of the *M. semimembranosus* (SM).

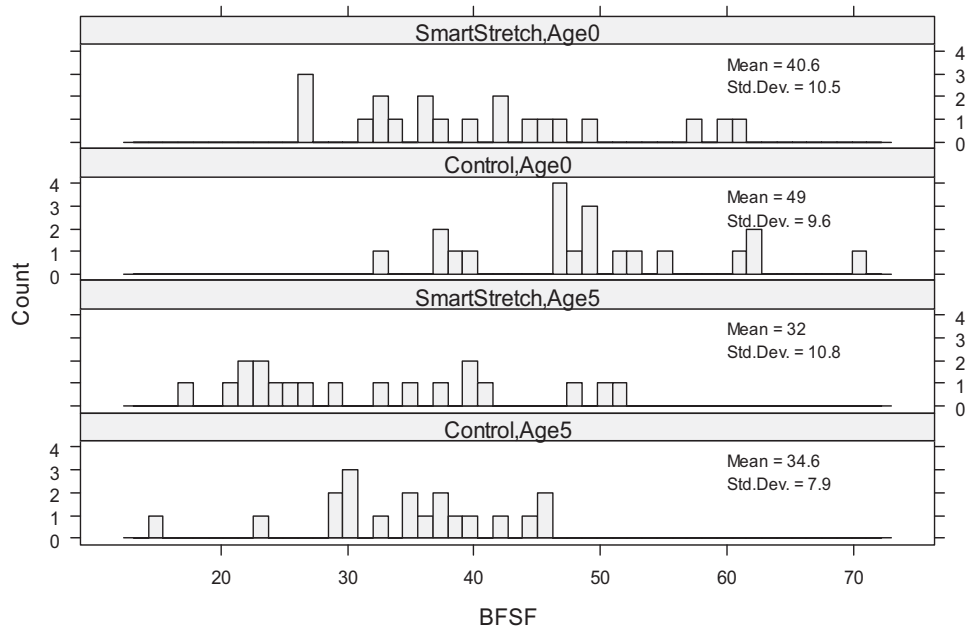


Figure 4.2. Histogram showing the effect of SmartStretch™ and control treatment at 0 and 5 days on shear force (N) distribution of the *M. biceps femoris* (BF).

4.3.3 Cooking loss and purge loss

Both stretch ($P < 0.05$) and ageing ($P < 0.05$) significantly affected cooking loss of the *M. biceps femoris*, but there was no significant interaction ($P > 0.05$) between the two treatments (Table 4.4). The cooking loss of the stretched *M. biceps femoris* was lower than non-stretched *M. biceps femoris* with means of 15.6 (se \pm 0.79) and 17.4 (se \pm 0.79) respectively. The 0 day aged *M. biceps femoris* had a lower cooking loss (15.5 se \pm 0.77) than 5 day aged *M. biceps femoris* (17.4 se \pm 0.77).

Table 4.4. Wald-statistic F -ratio for effects stretch, age, stretch x age on cooking loss % for *Mm. semimembranosus* (SM) and *biceps femoris* (BF).

Terms	Cooking loss SM (%)		Cooking loss BF (%)	
	df	F -ratio	df	F -ratio
Stretch	1,61	1.69 ^{ns}	1,59	5.50*
Age	1,65	4.25*	1,61	5.38*
Age x Stretch	1,37	4.11 ^{ns}	1,37	2.04 ^{ns}

* $P < 0.05$, ^{ns} $P > 0.05$.

Cooking loss results for the *M. semimembranosus* indicated there was no significant difference ($P > 0.05$) between SmartStretch™ and control treatments (Table 4.4) with means of 19.8 (se \pm 0.75) and 20.9 (se \pm 0.75) respectively. There was however a significant ageing effect ($P < 0.05$) in the *M. semimembranosus*, but this effect was the converse to that of the *M. biceps femoris* with 0 day aged samples having greater cooking loss when compared to the controls 21.2 (se \pm 0.62) and 19.6 (se \pm 0.63). In addition there was no interaction ($P > 0.05$) between stretch and ageing treatments (Table 4.4).

The overall purge was measured on the whole leg and stretch treatment resulted in significantly greater ($P < 0.001$) purge loss when compared to the control 1.82% (se \pm 0.07) and 1.61% (se \pm 0.07) respectively (*Wald statistic* $F(1,28)=16.4 P=0.001$).

4.3.4 Sarcomere Length

The results in Table 4.5 show that there was a significant difference ($P < 0.05$) between SmartStretch™ treatment and control for both the *Mm. semimembranosus* (Wald Statistic $F(1,27)=6.32$ $P=0.018$) and *semitendinosus* (Wald statistic $F(1,19)=15$ $P=0.001$) sarcomere length at 0 days of ageing.

Table 4.5. Predicted means (s.e.) of *Mm. semimembranosus* (SM) and *semitendinosus* (ST) sarcomere length (μm) according to treatment.

	Sarcomere length (μm)	
Muscle	SmartStretch™	Control
SM	1.82 (0.05) a	1.61 (0.04) b
ST	2.12 (0.07) a	1.84 (0.03) b

Means followed by a different letter in a row (a, b) are significantly different ($P < 0.05$).

The impact of stretch treatment on the variation or distribution of sarcomere length for both the *Mm. semimembranosus* and *semitendinosus* is shown in Figures 4.3-4.4 respectively. This showed that there was a greater variation in the SmartStretch™ treatment for sarcomere length of both muscles.

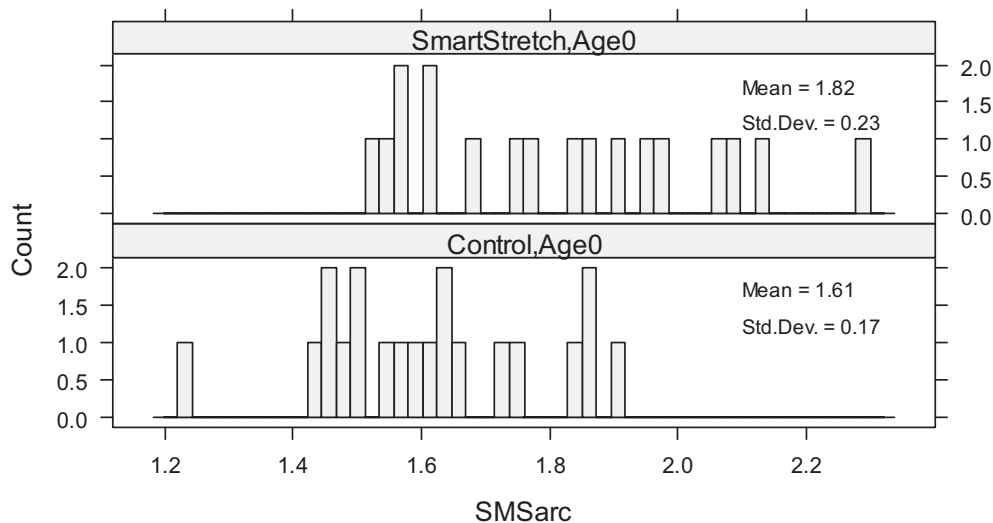


Figure 4.3. Histogram showing the effect of SmartStretch™ and control treatment at 0 days on *M. semimembranosus* sarcomere length (μm) (SMSarc) distribution.

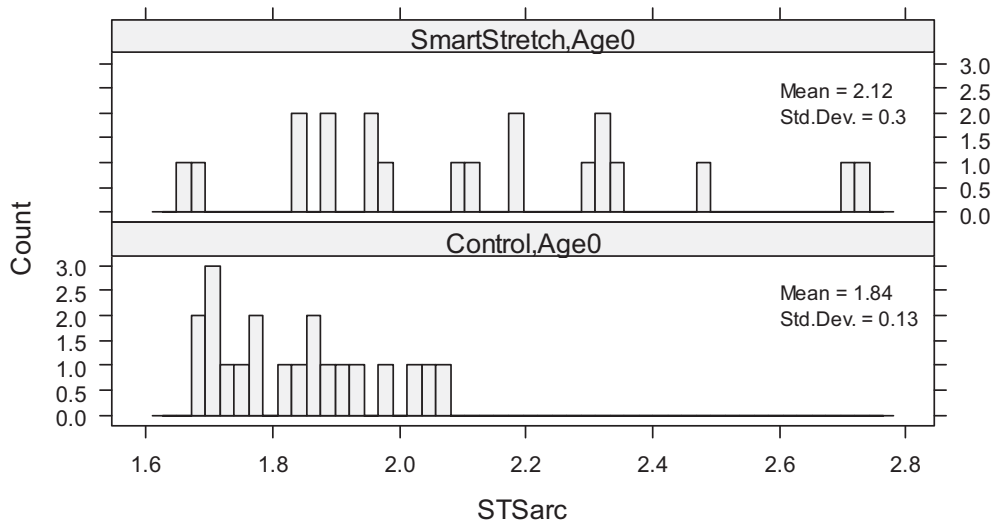


Figure 4.4. Histogram showing the effect of SmartStretch™ and control treatment at 0 days on *M. semitendinosus* (ST) sarcomere length (μm) (STSarc) distribution.

4.3.5 Histology

Samples were taken from the *M. semimembranosus* to examine the structure of the myofibres and a sample of images is shown below. In Figure 4.5 a major break across a fibre is shown.

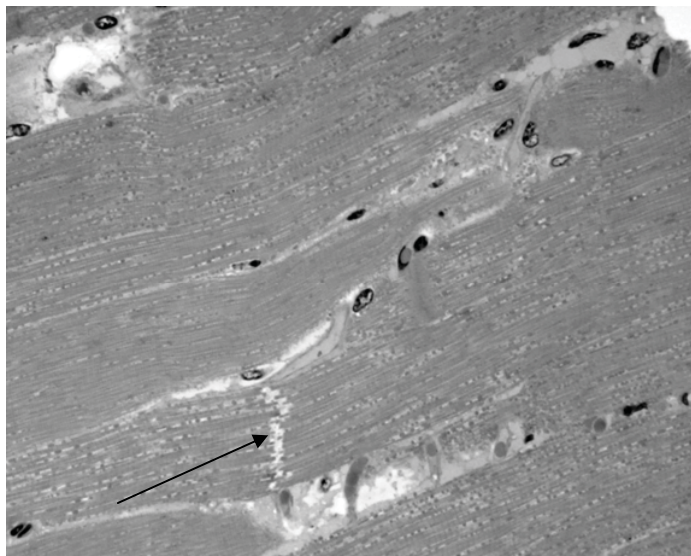


Figure 4.5. Image of muscle fibres with a major break across the fibre indicated. Muscle came from non-stretched *M. semimembranosus* after 5 days of ageing. 40x magnification.

An example image from muscle subjected to the SmartStretch™ with no ageing is shown in Figure 4.6. This shows a wavy pattern indicative of fibres under pressure. These waves combined with bent fibres were also counted and termed distorted fibres.

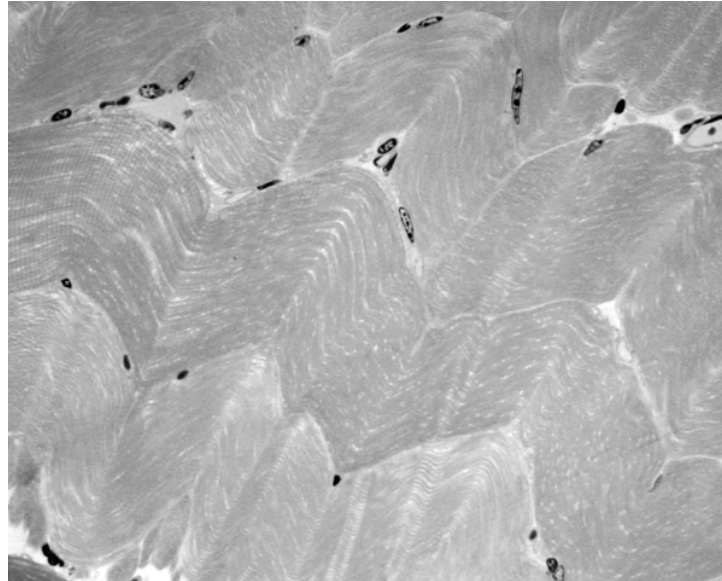


Figure 4.6. Image of muscle fibres showing a wave pattern. Muscle came from a stretched *M. semimembranosus* after 0 days of ageing. 40x magnification.

Table 4.6 shows the significance of treatment effects on the percentage of fibre breaks, percentage of distorted fibres and particle size.

Table 4.6. Wald statistic, *F*-ratio for effects stretch, age and stretch x age on the percentage of breaks in fibres (fibre break), percentage of either wavy or bent fibres (distorted fibres) and particle size.

Terms	Fibre breaks (%)		Distorted fibres (%)		Particle size (µm)	
	df	<i>F</i> -ratio	df	<i>F</i> -ratio	df	<i>F</i> -ratio
Stretch	1,36	1.23 ^{ns}	1,74	0.109 ^{ns}	1,59	7.07 ^{ns}
Age	1,50	33.6***	1,75	110.0***	1,62	3.88***
Age x Stretch	1,33	1.59 ^{ns}	1,39	2.06 ^{ns}	1,40	2.00 ^{ns}

*** $P < 0.001$; ^{ns} $P > 0.05$.

There was no effect of stretching on myofibrillar degradation of the *M. semimembranosus* measured as breaks in fibres, but there was a significant ($P < 0.001$) effect of ageing, such that as ageing time increased so did the number of fibre

breaks (Table 4.7). There was no interaction ($P > 0.05$) between stretching and ageing. There was also no effect of stretching on the distortion of fibres, but again a significant effect of ageing ($P < 0.001$) with a reduction in distortion due to ageing (Table 4.7). There was no interaction between stretching and ageing.

Table 4.7. Predicted means (s.e.) for the percentage of breaks in fibres and the percentage of either wavy or bent fibres (distorted) for the *M. semimembranosus* according to treatment groups.

Treatments	Fibre breaks (%)	Distorted fibres (%)
<i>Stretching</i>		
Stretch	13.5a	45.8a
Control	19.0a	47.0a
<i>Ave S.E.D.</i>	<i>4.41</i>	<i>4.33</i>
<i>Ageing</i>		
0 day aged	4.0 a	70.6b
5 day aged	28.5b	22.2a
<i>Ave S.E.D.</i>	<i>4.41</i>	<i>4.33</i>

Means followed by a different letter within rows and columns for a trait are significantly different $P = 0.05$.

4.3.6 Particle Size

The SmartStretch™ treatment had no effect on myofibrillar degradation of the *M. semimembranosus* which was measured using particle size analysis. There was a significant difference ($P < 0.001$) between ageing treatments. This difference showed that 0 day aged samples had a predicted mean particle size of 190µm and after 5 days ageing it was 140µm with average standard error of difference of 8.3µm.

4.3.7 Regression Analysis

To determine whether the stage of *rigor* impacted on shear force initial pH was included into the model and was determined not to be significant ($P = 0.13$). To examine the relationship between *M. semimembranosus* shear force (SMSF) and *M. semimembranosus* sarcomere (SMSarc) on 0 day aged samples a bi-variate mixed

model analysis was performed. This allowed different means for each trait within each level of stretch, correlated random effects for each trait across carcasses and correlated residuals. The results indicated that there was no significant ($P > 0.05$) correlation at either the carcass or the residual level (Figure 4.7).

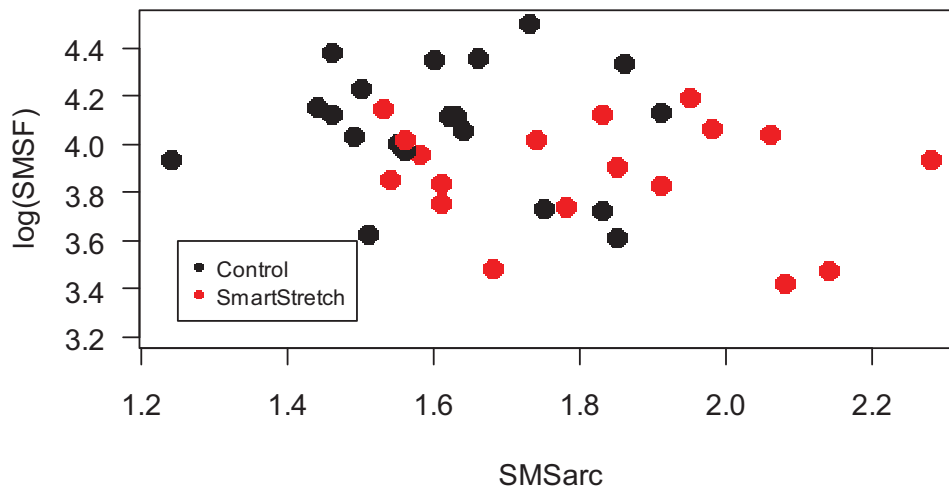


Figure 4.7. Relationship between the *M. semimembranosus* shear force (logSMSF) and *M. semimembranosus* sarcomere length μm (SMSarc) according to treatment for 0 day aged samples.

In addition to this there was no significant relationship ($P = 0.35$) shown between *M. semimembranosus* initial pH and *M. semimembranosus* sarcomere length. The percent increase in muscle length did not show a significant relationship with sarcomere length ($P = 0.72$) or shear force ($P = 0.50$). There was however a significant relationship ($P < 0.05$) between the percentage of fibre breaks and *M. semimembranosus* shear force, such that as the percentage of fibre breaks increased the shear force decreased. There was also no relationship between fibre breaks and particle size ($P = 0.48$).

4.4 Discussion

In the present study the SmartStretch™ treatment was effective in reducing shear force after 0 days of ageing in both the *Mm. biceps femoris* and *semimembranosus*. This was despite the fact that due to the application of electrical stimulation the muscles were on an accelerated path to *rigor* (Hwang et al. 2003a). Based on the initial pH and temperature, of the *Mm. semimembranosus* and *semitendinosus*, were on average still in the *pre-rigor* phase. Additionally there was no significant effect found when initial pH was added as a covariate in the model for *M. semimembranosus* shear force, indicating that although some muscles may have been in stages of *rigor* they had not completed *rigor mortis* the term that refers to when muscles are physically stiff (Hwang et al., 2003), hence enabling the stretch treatment to have an effect.

In the present study SmartStretch™ caused a 13% (5.5N) reduction in *M. biceps femoris* shear force irrespective of ageing treatment. As expected as ageing time increased shear force results decreased. The benefits were also shown in shear force results for the *M. semimembranosus* where SmartStretch™ caused a 15.2% (9.2N) reduction in shear force at 0 days of ageing. However, after 5 days of ageing the benefits of SmartStretch™ were nullified. It appears the benefits from the SmartStretch™ treatment were not as profound when applied to a whole leg where multiple muscles are involved as compared to an individual *M. semimembranosus* as shown in Chapter 3. This is most likely due to the fact that when an individual muscle is processed, such as the *M. semimembranosus*, it is easy to ensure that muscle fibres are aligned longitudinally in reference to the rubber sleeve inside the SmartStretch™ machine thus ensuring the stretch potential of the muscle is achieved. In addition to

this, all pressure applied by the SmartStretch™ machine is solely received by the individual muscle.

Previous work by Thompson et al. (2005) on both lamb and mutton *M. biceps femoris* indicated that significant improvements in subjective tenderness could also be achieved by Tenderstretching. These benefits are likely to be a result of the improvements achieved in sarcomere length due to the increased tension placed on muscle fibres by stretching hence inhibiting the physical ability of muscle fibres to contract Bouton et al. (1973b).

It is evident in the current study that the SmartStretch™ treatment is an effective tool in controlling muscle contraction given the sarcomere length increased from 1.61 to 1.82 μm and 1.84 to 2.12 μm for both the *Mm. semimembranosus* and *semitendinosus* muscles respectively. These results support the findings reported by Troy (2006) where meat wrapping using the Pi-Vac Elasto Pack System® was found to be effective in controlling sarcomere shortening. This wrapping method is a similar concept to the SmartStretch™ as the aim is to restrain and potentially stretch the deboned muscle to prevent the muscle fibres contracting. However work by Devine et al. (2002b) where samples were hand wrapped using polyethylene cling film 11 μm thick, indicated that the wrapping method employed by Devine et al (2002b) only stopped the muscle from shortening rather than applying stretch. Results presented in Chapter 3 which examined the effect of SmartStretch™ treatment on the *M. semimembranosus* also support the results of the current study with an increase in sarcomere length from 1.54 to 2.19 μm when the SmartStretch™ treatment was applied. It is apparent that on average SmartStretch™ in Chapter 3 was able to achieve greater difference in sarcomere length than in the current study. It could be concluded that based on the percentage increase in length, on average the

semimembranosus muscle in Chapter 3 exhibited greater stretch. However, results in the current study show the changes in the percentage increase in length are not reflected by those in the sarcomere length and this outcome is supported by results in Chapter 3. This indicates that other factors are impacting on sarcomere length. There was greater variation in sarcomere length exhibited for SmartStretch™ treated samples. It was concluded by Hopkins et al. (2000a) that the greater variation in sarcomere length for Tenderstretched samples was caused by the disrupted Z disks, but this is unlikely to be the explanation for the effect found in the current study.

Particle size (PS) analysis is one approach to measuring the degree of proteolysis that has occurred in meat by examining myofibrillar degradation (Karumendu et al., 2009). In the current study it was shown that myofibrillar degradation or proteolysis of the *M. semimembranosus* was not altered by SmartStretch™ and this outcome was consistent with the results in Chapter 3 and those of Devine et al. (1999) where the utilisation of SmartStretch™ or hand wrapping of meat respectively had no effect on proteolysis, but ageing did. Many studies (e.g. Chapter 3; Devine et al. 1999; Karumendu et al. 2009 & Koohmaraie et al. 1996) have shown that during ageing proteolysis is evident. The decline in particle size over time indicates that the fibres had degraded during ageing.

Simply, muscle is made up of contractile fibres which are attached to each other by connective tissue (Tornberg, 1996; Taylor & Frylinck, 2003). Given the degradation of collagen hence connective tissue *post-mortem* is limited to 1-5% total (Maltin et al. 2003) most of the changes that occur *post-mortem* are caused from the detachment of the endomysium, breaks in sarcomeres and fibre contraction (Taylor & Frylinck, 2003). It was concluded by Taylor & Frylick, (2003) that both fibre detachment and fibre breaks affect meat tenderness. To gain a better understanding of

the impact that SmartStretch™ has on muscle structure, fibre histology was measured in the *M. semimembranosus*. The results confirmed that the SmartStretch™ treatment did not impact on the number of fibre breaks or fibre distortion and thus did not accelerate proteolysis consistent with the particle size results. The significant relationship shown between fibre breaks and shear force in the current study supported work by Taylor & Frylinck (2003) and Martin, Hopkins, Gardner, & Thompson (2006). However it was of interest that the distortion of fibres decreased significantly with ageing as previous work by Taylor & Frylinck (2003) showed that there was no real effect of ageing and concluded that this may be an effect of slaughter. A possible explanation for this difference could be that the fibres sampled at day 0 were not fully in *rigor* given that samples were collected and fixed in solution within approximately 2 hours of exsanguination and that by comparison the aged samples had been able to fully enter *rigor* leading to a straightening of fibres.

Cooking loss was less for *M. biceps femoris* stretched muscle which is consistent with *M. semimembranosus* in Chapter 3. However in the current experiment, the stretch treatment had no effect on cooking loss of the *M. semimembranosus*. Similar to the findings where shown by Devine et al. (2002a) and Toohey et al. (2008a) where wrapping and ageing hot-boned sheep *M. longissimus* had no effect on cooking loss percent. A possible explanation for this could be due to the fact that the previous work by Devine et al. (2002b) and Toohey et al. (2008a) only constricted the muscle with the wrapping technique employed and did not stretch the muscle. Given that in the current study the degree of stretch was less than that shown in Chapter 3 this may explain the similar findings to Devine et al. (2002b) and Toohey et al. (2008a). There was a contrast between treatments across muscles for ageing in the current study, such that *M. biceps femoris* 0 day aged had a lower cooking loss compared to 5 day aged

samples and the reverse was found for the *M. semimembranosus*. Devine et al. (2002b) also showed that cooking loss decreased as ageing period increased although it was not shown to be significant. This decrease in cooking loss as ageing time increases could support the theory reported in early work by Bouton et al. (1972); Bouton et al. (1973a, 1973b) that stretched muscles had greater water holding capacity resulting in less water loss during subsequent ageing and finally cooking. Given that greater stretch was achieved in the results presented in Chapter 3 and neither result in the current study is supported by outcomes in Chapter 3 the outcomes are inconclusive.

Anecdotal evidence based on observations in Chapter 3, suggested that there were varying amounts of initial purge and it was speculated that the SmartStretch™ treatment may have lost more initial purge. Hence in the current experiment the percentage of purge lost after 5 days of ageing was measured and it confirmed a greater percentage of purge loss in the SmartStretch™ treatment compared to the control. This could potentially nullify the differences found in cook loss. Thus the SmartStretch™ treatment does not appear to have any significant effect on overall water holding capacity of the end product.

Given that on average after SmartStretch™ treatment was applied a 14% increase in leg length and a 45% decrease in circumference occurred it is clear that the SmartStretch™ treatment transformed the overall shape of the leg, which was consistent with the results presented in Chapter 3. Although the results from Chapter 3 did show a greater increase in length of 24% the results in the current experiment had a greater decrease in circumference of 45% as opposed to 24%. This is most likely due to the more complex multiple muscle structure of the hindleg compared to a single muscle. The transformation of the sheep leg is an important industry outcome

as to our knowledge there is no other machine that can take a whole sheep leg and shape it to a consistent size and shape. As outlined in Chapter 3 the ability to produce a consistent shape is considered a desirable trait by the food service industry (Tarrant, 1998) irrespective of any potential tenderness benefits.

4.5 Conclusions

The results presented in this study have highlighted the potential of SmartStretch™ for improving specifically the tenderness of whole hot boned hind legs. However this benefit was diluted compared to stretching individual muscles as reported in Chapter 3. Based on shear force and sarcomere results it can be concluded that the SmartStretch™ treatment was successful in preventing the unrestrained muscle from shortening. These fibres could have been physically disrupted by this treatment, but the histology results did not suggest that SmartStretch™ causes more distorted fibres. Both the particle size and histology results indicate that the SmartStretch™ does not cause the any acceleration of proteolysis. Given there was no relationship between shear force and sarcomere length this indicates that other mechanisms are impacting on the variation in shear force and a possible explanation is that the SmartStretch™ treatment is altering muscle structure or connective tissue.

Chapter 5: The impact of medium voltage stimulation and SmartStretch™ technology on sheep topside (*M. semimembranosus*) meat quality traits under commercial processing conditions

5.1. Introduction

Consumer satisfaction is a major factor affecting repeat purchasing of any product (Lorenzen et al. 2003). Visual appearance and overall eating quality are two main factors which influence consumer choice to purchase and repurchase red meat (Maltin et al. 2003; Thompson, 2002; Egan, et al. 2001). In a recent review paper by Mancini & Hunt, (2005) it was stated that ‘meat purchasing decisions are influenced by colour more than any other quality factor because consumers use discoloration as an indicator of freshness and wholesomeness’. This has been shown by earlier work to have a negative economic impact on the meat industry (Smith et al. 2000). Meat colour and tenderness are both influenced by many *post-mortem* factors, for example electrical stimulation, hanging method (Achilles tendon or Tenderstretch), boning technique (cold or hot boned), chilling regime, storage and packaging (Maltin et al., 2003; Thompson, 2002; Tornberg, 1996; Hopkins & Geesink, 2009; Devine et al. 2004; Mancini & Hunt, 2005; Monin, 2004; Kropf, 2003). Hence it is critical that these two traits are not compromised in the quest for accelerating the processing of meat.

Hot boning is a technique used to accelerate the processing of meat from a carcass into a carton (Pisula & Tyburcy, 1996). However as highlighted in Chapter 3 there are many advantages and disadvantages associated with this method. To combat some of the negative effects of hot boning such as muscle shortening hot boning is almost always performed in conjunction with electrical stimulation. The role of

electrical stimulation is to hasten the onset of *rigor* (Hwang et al. 2003a) and hence reduce the degree of muscle shortening when the muscles are removed from the skeletal constraint. Macfarlane, Harris, & Shorthose (1974) highlighted that another approach to preventing muscle shortening is to restrain muscles physically until they are in *rigor mortis*. Many studies such as Locker (1960); Herring et al. (1967); Davey et al. (1967); Devine et al. (2002b) and Troy (2006) all proved the concept that *pre-rigor* excised muscles could be stretched in various ways which resulted in improved tenderness.

The results outlined in Chapters 3 and 4 showed that the stretching prototype device (licensed as SmartStretch™) significantly improved sheep meat tenderness and significantly increased sarcomere length when processed under commercial conditions. This meant that all carcasses were exposed to a number of electrical inputs including immobilisation; electronic bleed and medium voltage electrical stimulation (Toohey, Hopkins, & Lamb, 2008b). Despite these electrical inputs it was concluded based on the results outlined in Chapters 3 and 4 that on average the muscles examined were still in a *pre-rigor* state when subjected to the stretching treatment hence improvements in shear force were achieved. However, based on results in Chapters 3 and 4 the variation around the mean for these data indicates that some muscles would have entered the *rigor* phase. This begs the question could application of electrical stimulation to achieve a lower pH be counterproductive to the effectiveness of SmartStretch™ treatment as early entry of *rigor mortis* would prevent effective stretching?

Therefore the aim of this study was to evaluate the interaction between medium voltage electrical stimulation, stretching and ageing on key meat quality traits

including meat tenderness and meat colour of hot boned sheep m. *semimembranosus* using a stretching prototype device SmartStretch™.

5.2. Materials and Methods

5.2.1 Animals

For testing the effect of the medium voltage stimulation, SmartStretch™ prototype machine and *post-mortem* ageing, a total of 80 sheep of mixed sex (ewes and wethers) were randomly selected from various consignments over two days. The sheep used from these different consignments were of varying backgrounds representing the typical animals processed by the abattoir. All sheep used in this experiment were classified as mutton (Anonymous, 2005), in that the animals were over 10 months of age and had more than two permanent incisors.

5.2.2 Experimental Design

The experiment was designed as a blocked split-plot experiment examining three treatments including; medium voltage electrical stimulation (carcasses were either stimulated or not stimulated), stretch (topside were either stretched using SmartStretch™ or not stretched) and ageing (samples were either aged for 0 or 5 days). Eighty animals were randomly selected and killed over two days with 40 carcasses processed each day, within each kill day, 20 carcasses were randomly allocated to stimulation and 20 carcasses to the non stimulated treatment group. Both the right and left topsides (*Mm. semimembranosus*, *adductor* & *gracilis*) HAM. 5073 (Anonymous, 2005) from each carcass were collected and each pair of topsides were assigned to stretch, ageing and stimulation treatment, resulting in eight treatment combinations including; Stimulation + 0 days ageing + SmartStretch™, Stimulation +

0 days ageing + control, Stimulation + 5 days ageing + SmartStretch™, Stimulation + 5 days ageing + control, No Stimulation + 0 days ageing + SmartStretch™, No Stimulation + 0 days ageing + control, No Stimulation + 5 days ageing + SmartStretch™ and No Stimulation + 5 days ageing + control. In addition, allocations to either one of 20 cooking batches were made for shear force samples.

5.2.3 Treatments and sample collection

The carcasses were processed under the normal commercial procedures of the abattoir. All animals were exposed to the high frequency immobilisation unit, applied for 25-35 secs (2000 Hz, 400 volts, and a maximum current of 9 amps over 7 animals, pulse width of 150 microseconds), moderate frequency immobilisation (800 Hz, 300 peak volts, a constant current of 1.7 amps, pulse width 150 microseconds) applied for 5-7secs and low voltage electronic bleed (15 Hz, 550 peak volts, constant current of 0.8 amps, pulse width 500 microseconds) applied for 20 seconds. Normally, all carcasses are also exposed to a post dressing medium voltage electrical stimulation system. However, in the current experiment carcasses were randomly allocated to one of two stimulation treatments. Half of the carcasses (n = 40) were exposed to post dressing medium voltage electrical stimulation (MVS) with a constant current 1.0 amp and pulse width of 2500 microseconds, but variable frequency across the 6 electrodes (the frequency for electrodes 1 & 2 was set at 25 Hz, 3 & 4 at 15 Hz and 5 & 6 at 10 Hz, with 300 peak volts) applied for 30-35 seconds. This treatment was applied within approximately 40 minutes of death and the other half of the carcasses (n = 40) were not stimulated.

Once stimulation treatments were applied, carcasses passed through a drying room for approximately 35 minutes with an average temperature of 8°C. Following this

both the right and left topsides (*Mm. semimembranosus, adductor & gracilis*) HAM. 5073 (Anonymous, 2005) from 80 carcasses (n = 160) were hot boned, collected and trimmed removing all fat and placed into their predetermined stretch and ageing treatments within 2 hours of death. The stretched samples were stretched using the SmartStretch™ machine as previously described in section 3.2.6. The 0 day aged samples were placed in a freezer set at -22°C within 4 hours of death. The 5 day aged samples were vacuum packed and chilled at 3-4°C for 5 days, cut into laboratory test subsamples and then frozen at -22 °C until testing.

5.2.4 Sample preparation

The 0 day aged topsides were tempered at an average temperature of 21.2°C for approximately 2 hours to allow the *Mm. adductor* and *gracilis* to be dissected from the *M. semimembranosus*. Then sarcomere, shear force, final pH and meat colour samples were cut from the *M. semimembranosus* whilst the *M. semimembranosus* was still predominately frozen. After 5 days of ageing at 3-4 °C the *Mm. adductor* and *gracilis* were dissected from the *M. semimembranosus* and samples cut into test subsamples for shear force, final pH and meat colour and then frozen at -22 °C until testing.

5.2.5 Muscle measurement, initial pH & temperature, final pH, sarcomere length, Warner Bratzler shear force and cooking loss

These measurements were taken and conducted as described in sections 3.2.4, 3.2.5, 4.2.9, 3.2.8, 3.2.9 and 3.2.10 respectively. Sarcomere length was measured on 0 day aged samples only.

5.2.6 Colour stability

Retail colour display was examined on both 0 and 5 day aged samples. For the 0 day aged product, a frozen slice of *M. semimembranosus* (3 cm thick) was taken from each sample, placed in plastic zip lock bags on trays and allowed to thaw overnight in a chiller set at 3-4°C. The following day a fresh surface was cut on each sample and they were placed individually on black foam trays (13.5 cm x 13.5 cm) and overwrapped with PVC food film wrap (15 µm thickness). After a blooming period of 30-40 min, initial colour values (L*, a*, and b*) and the reflectance ratio of 630/580 nm, was measured with a Hunter Lab meter (Model 45/0-L) with an aperture size of 25 mm. The reflectance ratio of 630/580 nm was suggested by Hunt (1980) as an indication of the formation of metmyoglobin. The Hunter Lab was calibrated with black and white tiles using Illuminant D-65, with 10 degree standard observer. Samples were displayed in the chiller under lighting (1000 lux) and colour was measured once a day for 4 days (final colour value). Each sample was measured twice at each measurement time and the values averaged. For the 5 day aged samples a 3 cm thick slice was taken fresh after the 5 day ageing period and these samples prepared and measured as described for the 0 day samples.

5.2.7 Purge percentage loss

Purge loss was determined on the 5 day aged samples. The weight of the samples and packaging was taken at collection. Just before freezing the weight of the samples (patted dry) and the weight of the packaging were taken. Purge loss percentage was then calculated using the following formula:

$$\text{Purge loss (\%)} = (\text{Initial weight} - \text{final weight} - \text{packaging weight}) / (\text{initial weight} - \text{packaging weight}) * 100$$

5.2.8 Statistical analysis

Linear mixed models (LMM) using restricted maximum likelihood (REML) with the statistical package ASReml (Gilmour et al. 2006) via the statistical package *asreml* (Butler, 2009) under R (R Development Core Team, 2009) were used to analyse the data. Variance components of the model were tested for significance using a likelihood ratio test whilst fixed effects were tested using Wald-related test statistics developed by Kenward & Roger (1997). These Wald-related test statistics have been developed to accommodate small sample inference. The degrees of freedom (df) are rounded down to be conservative. Generalized LMM's (GLMM), e.g. logistic regression, are handled in ASReml using a Schall linearization approach (Schall, 1991), with tests for significance of random and/or fixed effects as for LMM's.

The model fitted for initial pH, initial temperature, shear force, cooking loss % and final pH had fixed effects for the treatments, electrical stimulation (levels = NoStim and Stim), stretch (levels = NoStretch and Stretch) and ageing (levels = 0 and 5 days) and the interaction effects between these treatments. Random effects included were effects for kill day (levels = kill day 1 and kill day 2), carcass within kill day (forty carcasses were included in each kill day), Cooking batch (was included for shear force and cooking loss percent) and random error. For initial pH initial temperature was included in the model. For sarcomere length there are no age effects in the fixed effects part of the model as sarcomere length was only measured on 0 day aged samples. In addition, as there was only a single result for each carcass, the carcass effect was removed from the random effects in the model (carcass confounded with random errors).

The 0 day and 5 day aged colour traits (L, a*, b* and ratio values 630nm/580nm) were each analysed separately. To facilitate the analysis of this data the independent variable TFFR (Time, in days, from first reading) and the dependent variables (L*, a*, b* and ratio values 630nm/580nm) were transformed as follows;

$$x = \log(\text{TFFR} + 1)$$

$$Y = \log(\log(\text{dependent variable}))$$

Both dependent and independent variables were transformed as shown above in order to linearise the relationship between the two variables. The model fitted to the above transformed data in each case is the random regression model.

$$Y = \text{baseline} + \text{stimulate} + \text{stretch} + \text{stimulate:stretch} + x + \text{stimulate:x} + \text{stretch:x} + \text{stimulate:stretch:x} + \textit{carcase} + \textit{carcase:x} + \textit{read} + \textit{stimulate:read} + \textit{stretch:read} + \textit{stimulate:stretch:read} + \textit{error}.$$

Here *read* corresponds to a factor with four levels, one for each unique reading time. Terms in bold/italic are fitted as random effects. The random regression parameters for carcasses are independent across carcasses, but are correlated within carcasses.

Based on the estimated regression parameters, associated variation of these estimates and the estimates of the variance parameters of the model, Monte Carlo methods were used to obtain estimates of the means and standard errors for the dependent variables, for given treatment levels, across time.

5.3 Results

5.3.1 Muscle measurements and pH characteristics

Stimulation treatment had no significant effect ($P > 0.05$) on either percent increase in length or percent decrease in circumference measure at site 1 or site 2. A statistical summary of the muscle measurements taken from the SmartStretch™ treatment according to stimulation treatment is shown in Table 5.1 to give an indication of the variance within the data.

Table 5.1 Mean, standard deviation (S.D.) and range for muscle dimensional measurements from stretch treatment across stimulation treatments.

Trait	Stimulation			No Stimulation		
	Mean	SD	Range	Mean	SD	Range
Increase in length (%)	30.8	7.09	15.6-46.9	29.9	7.02	11.8-46.9
Decrease in circumference 1 (%)	48.9	12.90	71.4-18.4	51.2	12.67	85.0 - 14.9
Decrease in circumference 2 (%)	18.1	9.51	41.4-6.3	20.8	12.16	47.6 - 11.9

There was no significant difference ($P > 0.05$) between stretch and ageing treatments nor were there any interactions for initial pH and initial temperature (Table 5.2). However initial temperature and stimulation treatment did have a significant effect on the initial pH (Table 5.2). As expected ageing treatment did have a significant effect ($P < 0.001$) on final pH, but there was no stretch, stimulation or interaction between any treatments (Table 5.2). There was no variation attributable to kill day indicating that animals were well balanced across the kill days.

Table 5.2. Wald-statistic *F*-ratio for effects of stimulation (Stim), stretch, age, stretch x stim, stretch x age, stim x age and stim x stretch x age on initial pH, initial temperature, final pH along with relevant covariate for initial pH.

Terms	Initial pH		Initial Temp (°C)		Final pH	
	df	<i>F</i> -ratio	df	<i>F</i> -ratio	df	<i>F</i> -ratio
Stim	1,77	37.89***	1,158	0.03 ^{ns}	1,76	5.09 ^{ns}

Stretch	-	-	-	-	1,76	2.48 ^{ns}
Age	-	-	-	-	1,76	1.22**
Age x Stretch	-	-	-	-	1,76	1.00 ^{ns}
Age x Stim	-	-	-	-	1,76	7.42 ^{ns}
Stim x Stretch	-	-	-	-	1,76	6.52 ^{ns}
Stim x Stretch x Age	-	-	-	-	1,76	1.07 ^{ns}
Initial temp	1,154	5.35*	-	-	-	-

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$, ^{ns} $P > 0.05$, term not used in model (-).

Initial pH analysis indicated that, for a given stimulation treatment, initial pH declined by 0.013 (s.e. \pm 0.006) units for each 1°C increase in initial temperature. At a given initial temperature, stimulated carcasses had an initial pH 0.17 (s.e. \pm 0.03) units lower than non stimulated carcasses. For example when the initial temperature was 32°C the non stimulated treatment had an initial pH of 6.27 (s.e. \pm 0.02) and the stimulated treatment was 6.10 (s.e. \pm 0.02). Results showed that the 5 day aged final pH was significantly lower at 5.80 (s.e. \pm 0.024) compared to 0 day aged samples which had a final pH of 5.85 (s.e. \pm 0.024).

5.3.2 Warner Bratzler shear force

Table 5.3 shows the significance of treatment effects and relevant covariates on shear force and cooking loss for the *M. semimembranosus*. Results showed that stimulation treatment had no significant effect ($P > 0.05$) on shear force either as a main effect or at individual combinations of stretch and ageing. There were significant differences ($P < 0.001$) found between both stretching and ageing treatments and there was also a significant interaction ($P < 0.001$) between these two traits as shown in Table 5.3.

Table 5.3. Wald-statistic F -ratio for effects stimulation (Stim), stretch, age, stretch x stim, stretch x age, stim x age and stim x stretch x age on shear force (N) and cooking loss (%) along with relevant covariate initial pH for shear force.

Terms	Shear force (N)		Cooking loss (%)	
	df	F -ratio	df	F -ratio

Stim	1,75	7.42 ^{ns}	1,76	5.60 ^{ns}
Stretch	^	^	1,76	6.00***
Age	^	^	1,76	7.40***
Age x Stretch	1,76	2.44***	1,76	1.54 ^{ns}
Age x Stim	1,76	2.44 ^{ns}	1,76	2.04 ^{ns}
Stim x Stretch	1,76	5.83 ^{ns}	1,76	7.38 ^{ns}
Stim x Stretch x Age	1,75	1.05 ^{ns}	1,76	9.31 ^{ns}
Initial pH	1,114	1.44 ^{ns}	-	-

*** $P < 0.001$; ^{ns} $P > 0.05$, ^ Not tested because there is a significant interaction, term not used in model (-).

The predicted means and standard errors for the shear force results are shown in Table 5.4. The interaction between stretching and ageing treatments was such that the 5 day aged SmartStretch™ group was the most tender and the 0 day no stretch (control) treatment was the toughest group.

Table 5.4. Predicted shear force means (N) and standard errors (s.e.) for stretch and ageing treatments.

Treatment	Shear force (N)	
	SmartStretch™	Control
0 day	46.6 (2.25) b	74.1 (2.25) c
5 day	38.3 (2.25) a	45.5 (2.25) b

Means having a following letter different are significantly different ($P < 0.001$).

The present study also examined the impact of treatments on the distribution of shear force. Figure 5.1, shows the variation in shear force for stretch, stimulation and ageing treatment groups. Based on the results in this figure it does not appear that any of the treatments consistently reduced the variation in shear force. However the 5 day aged, stretched and stimulated samples exhibited the least variation and the 0 day aged, stretch and non stimulated treatment showed the greatest variation. When stimulation and ageing treatments were dropped from the model overall SmartStretch™ reduced the variation in shear force when compared to control (means \pm S.D. SmartStretch™ 42 ± 9.3 N, Control 60 ± 17.6 N).

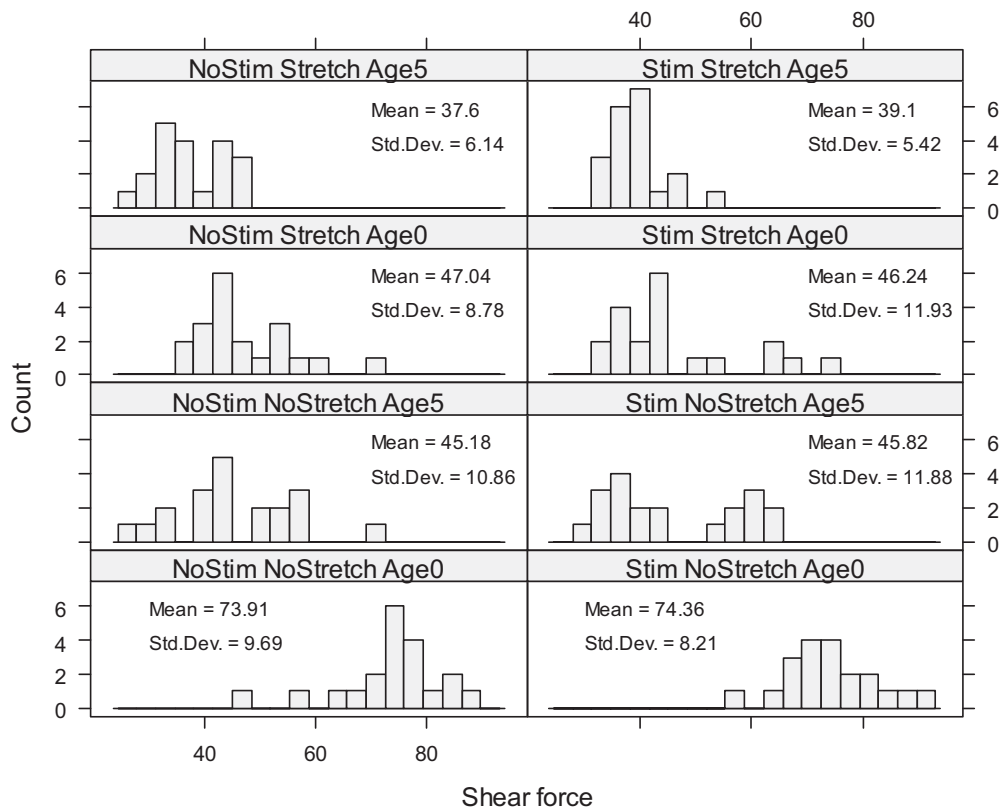


Figure 5.1. Histogram showing the effect of the stretch and stimulation treatments at 0 and 5 days on the distribution of shear force (N).

5.3.3 Cooking loss

There was a significant difference between stretch and ageing treatments ($P < 0.05$) for cooking loss as shown in Table 5.3, but stimulation treatment and the interaction between treatments had no impact on cooking loss ($P > 0.05$). These results showed that the no stretch (control) treatment had greater cooking loss 22.5% (s.e. ± 0.29) compared to the SmartStretch™ treatment 19.3% (s.e. ± 0.29) irrespective of ageing and stimulation treatment. The results also showed that the 5 day aged samples had greater cooking loss 22.6% (s.e. ± 0.29) compared to 0 day aged samples 19.2% (s.e. ± 0.29), irrespective of stretch and stimulation treatment.

5.3.4 Purge loss

There was a significant difference between stretch treatments ($P < 0.05$) for purge loss percent, but stimulation treatment or the interaction between any treatments had no impact on purge loss ($P > 0.05$). These results showed that the SmartStretch™ treatment had significantly greater purge loss compared to the control treatment 2.4% (s.e. ± 0.24) and 0.5% (s.e. ± 0.05) respectively (*Wald Statistic: $F(1,39)=124.8$ $P<0.001$*).

5.3.5 Sarcomere Length

Stimulation treatment had no significant ($P > 0.05$) effect on sarcomere length, but there was significant ($P < 0.001$) effect due to SmartStretch™ treatment. The sarcomere lengths were significantly increased by SmartStretch™ treatment compared to no stretch (control) 2.27 (s.e. ± 0.09) and 1.53 (s.e. ± 0.06) μm respectively (*Wald Statistic: $F(1,47)=86.6$ $P<0.001$*). The impact of treatments on the distribution of sarcomere length was examined. Based on Figure 5.2 the variation appears larger for the SmartStretch™ treatment compared to the control treatment, irrespective of stimulation treatment.

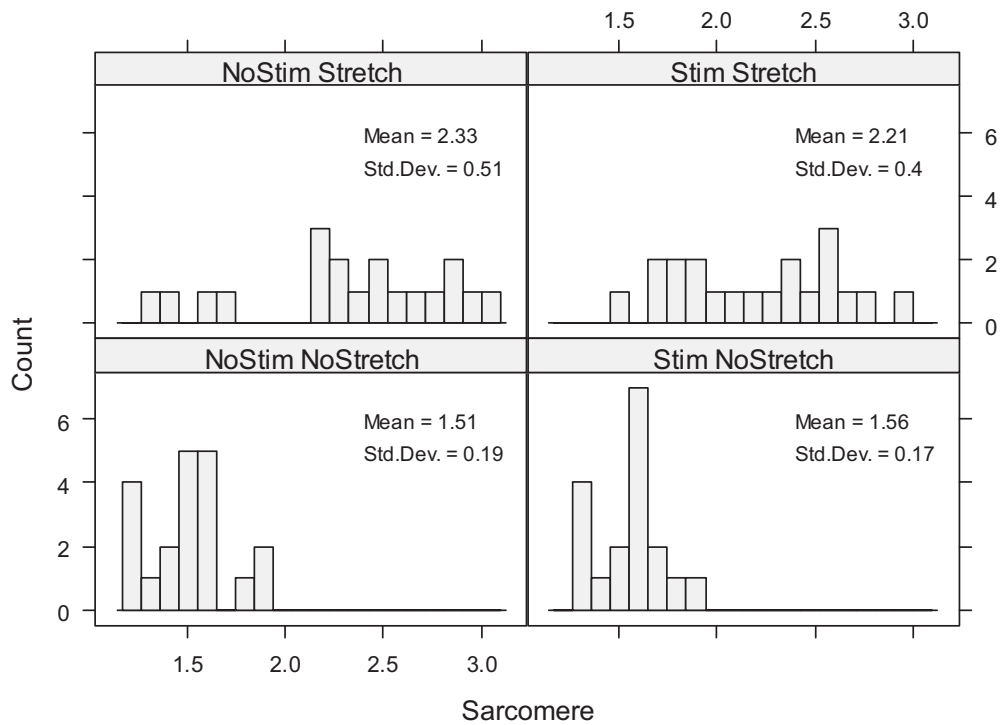


Figure 5.2. Histogram showing the effect of SmartStretch™ and no stretch treatment and stimulation level (Stim and NoStim) on the distribution of sarcomere length (μm).

5.3.6 Colour stability

A repeated measures analysis was performed on L*, a*, b* and ratio 630/580nm (indication of metmyoglobin formation) values for both 0 and 5 days aged samples, separately, to examine the impact of stimulation and stretch treatment on colour over time. Based on these analyses, stimulation had no effect ($P > 0.05$) on colour stability at either 0 or 5 days aged for any colour trait (Table 5.5 and 5.6).

Table 5.5. Wald-statistic *F*-ratio for effects stimulation (Stim), stretch, log of TFFR (X) and the interaction between stretch:stim, stretch:X, stim:X and stim:stretch:X on ratio 630/580nm a*, b* and L* values at 0 day aged.

	0 Day aged							
	Ratio 630/580nm		a*		L*		b*	
	df	<i>F</i> -ratio	df	<i>F</i> -ratio	df	<i>F</i> -ratio	df	<i>F</i> -ratio
Stim	1,76	0.92 ^{ns}	1,76	0.96 ^{ns}	1,73	0.41 ^{ns}	1,76	0.91 ^{ns}
Stretch	1,5	2.89 ^{ns}	1,25	14.23 ^{ns}	1,73	15.96**	1,34	7.28*
Stim:Stretch	1,76	0.17 ^{ns}	1,76	0.10 ^{ns}	1,76	3.66 ^{p=0.059}	1,76	3.24 ^{ns}
X	1,2	104.3**	1,19	240.9***	1,2	0.08 ^{ns}	1,2	0.01 ^{ns}
Stim:X	1,76	0.00 ^{ns}	1,76	0.03 ^{ns}	1,4	0.25 ^{ns}	1,76	0.79 ^{ns}
Stretch:X	1,5	5.85 ^{p=0.06}	1,19	4.24 ^{p=0.053}	1,4	0.35 ^{ns}	1,15	0.02 ^{ns}
Stim:Stretch:X	1,76	3.46 ^{ns}	1,76	3.21 ^{ns}	1,76	1.37 ^{ns}	1,76	3.15 ^{ns}

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; ^{ns} $P > 0.05$

Table 5.6. Wald-statistic *F*-ratio for effects stimulation (Stim), stretch, log of TFFR (X) and the interaction between stretch:stim, stretch:X, stim:X and stim:stretch:X on ratio 630/580nm a*, b* and L* values at 5 day aged.

	5 Day aged							
	Ratio 630/580nm		a*		L*		b*	
	df	<i>F</i> -ratio	df	<i>F</i> -ratio	df	<i>F</i> -ratio	df	<i>F</i> -ratio
Stim	1,9	0.07 ^{ns}	1,45	0.49 ^{ns}	1,74	0.01 ^{ns}	1,76	0.64 ^{ns}
Stretch	1,9	0.00 ^{ns}	1,45	1.02 ^{ns}	1,56	1.19 ^{ns}	1,8	0.94 ^{ns}
Stim:Stretch	1,9	3.39 ^{ns}	1,45	2.59 ^{ns}	1,74	3.33 ^{ns}	1,76	0.57 ^{ns}
X	1,2	99.07**	1,2	16.21 ^{p=0.056}	1,2	4.34 ^{ns}	1,2	0.10 ^{ns}
Stim:X	1,67	0.30 ^{ns}	1,47	0.04 ^{ns}	1,9	1.01 ^{ns}	1,76	0.38 ^{ns}
Stretch:X	1,67	3.57 ^{ns}	1,47	3.22 ^{ns}	1,3	0.02 ^{ns}	1,4	0.17 ^{ns}
Stim:Stretch:X	1,67	2.36 ^{ns}	1,47	1.74 ^{ns}	1,9	0.18 ^{ns}	1,76	0.86 ^{ns}

** $P < 0.01$; ^{ns} $P > 0.05$.

The ratio 630/580nm and a^* values at both 0 and 5 days showed significantly different trends with time between the stretched and control treatments, but with no significant difference at time zero. This interaction was such that the no stretch treatment ratio 630/580nm and a^* values at both 0 and 5 days decreased at faster rate when compared to the stretch treatment. This difference in decline is illustrated in Figures 5.3 to 5.6.

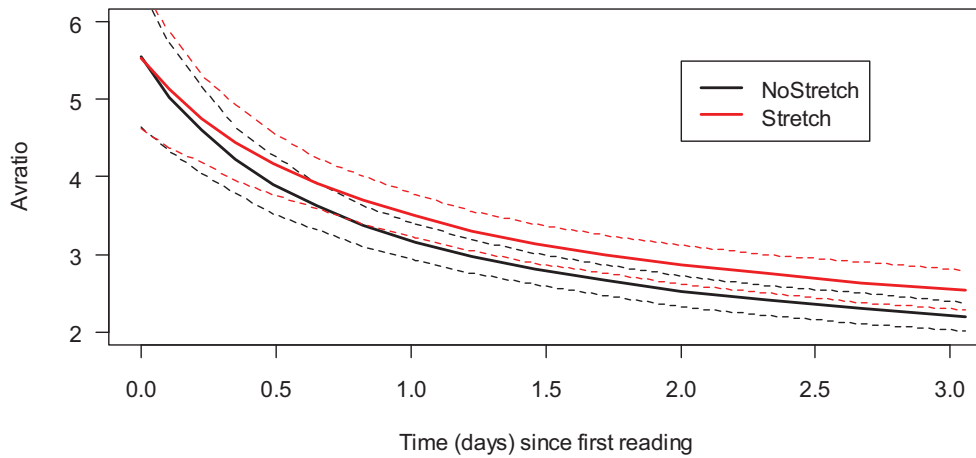


Figure 5.3. Predicted 0 day ratio 630/580nm values for stretch and no stretch treatments (s.e. ± 2) over time.

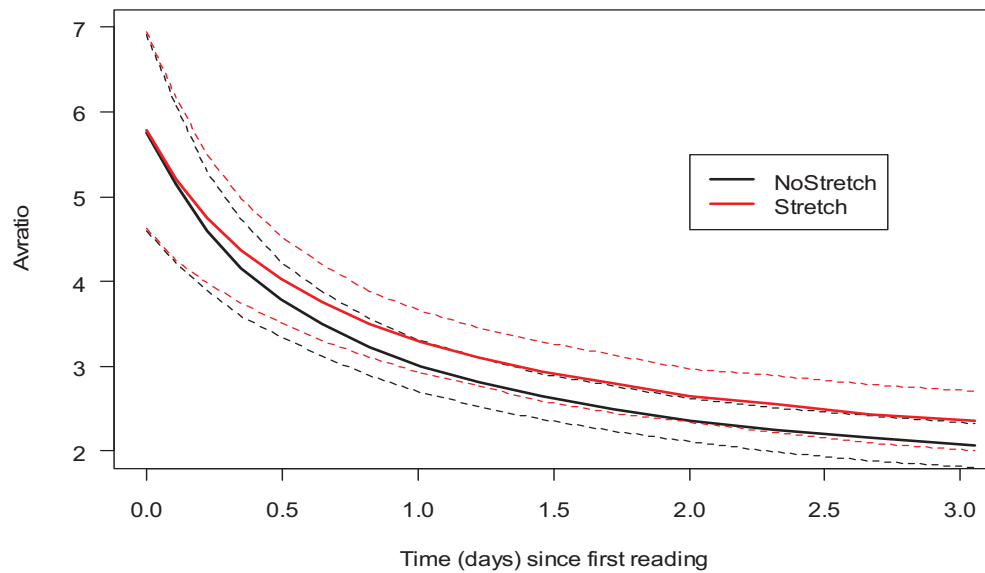


Figure 5.4. Predicted 5 day ratio 630/580nm values for stretch and no stretch treatments (s.e. ± 2) over time.

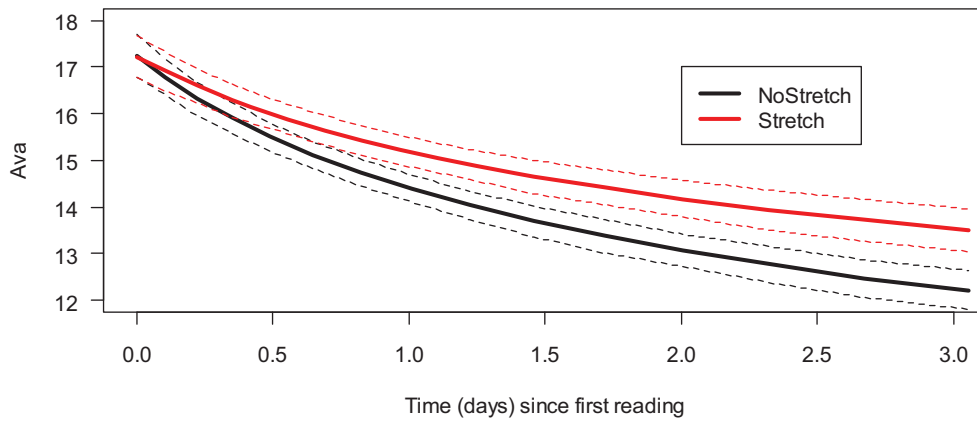


Figure 5.5. Predicted 0 day a^* values for stretch and no stretch treatments (s.e. ± 2) over time.

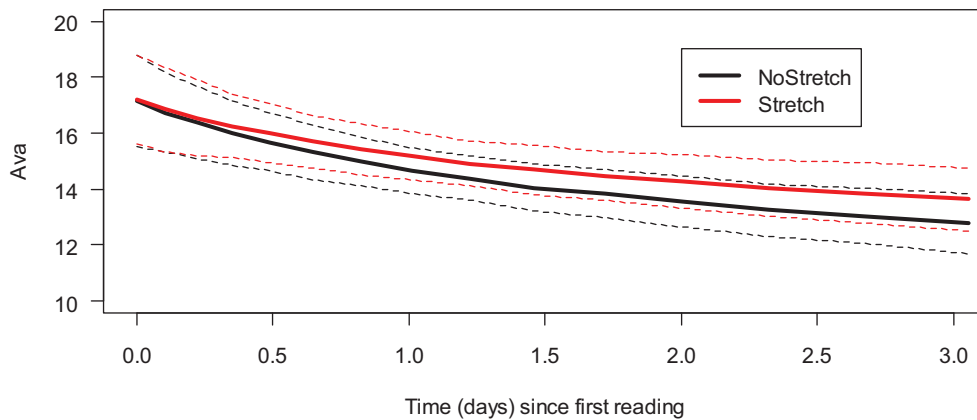


Figure 5.6. Predicted 5 day a^* values for stretch and no stretch treatments (s.e. ± 2) over time.

The analysis of b^* values for 0 day aged samples indicated that stretch treatment was the only significant effect (Table 5.6). It is estimated that stretch samples on average had a 0.66 (s.e. = 0.25) higher trait b value than NoStretch samples at each time point when colour was measured. The analysis of b^* values for 5 day aged samples indicated that there was no significant difference between treatments or any interactions between treatments.

5.4 Discussion

The present study showed that although medium voltage stimulation did have a significant impact on initial pH which was consistent with results by Toohey et al. (2008b), it did not provide any additional improvements in shear force when used in combination with the SmartStretch™ treatment. Many previous studies and reviews (Hwang et al. 2003a; Devine et al. 2004; Chrystall & Devine, 1978; Polidori, Lee, Kauffman, & Marsh, 1999; Pearce et al., 2006) have shown that the advantages of the use of various types of electrical stimulation in improving meat quality. Despite the drop in initial pH in the present study, on average, samples that were subjected to medium voltage stimulation were not in *rigor* when SmartStretch™ was applied. Given the carcasses were not in *rigor* there were no implications on the effectiveness of stretch treatment with regard to shear force or sarcomere length as shown.

Previous work by Pearce et al. (2009) showed contrasting results to the present study such that medium voltage stimulation improved shear force even after 30 days of ageing. In the current study all carcasses were exposed to a number of other electrical inputs including; high frequency immobilisation, moderate frequency immobilisation and low voltage electronic bleed, which may have nullified the shear force benefits of applying medium voltage stimulation. In the study by Toohey et al. (2008b) which was conducted at the same abattoir as the present study, three of the four electrical inputs used in the current experiment were compared. From this it was concluded that medium voltage stimulation when applied individually and in combination with moderate frequency immobilisation and or low voltage electronic bleed was effective in lowering initial pH when compared to moderate frequency immobilisation and or low voltage electronic bleed individually or in combination with each other. However shear force was not quantified to know if the initial pH

benefits would translate into tenderness benefits. Earlier work by Toohey & Hopkins (2007) did quantify that high frequency immobilisation did not have a significant effect on shear force.

Hence irrespective of stimulation treatment the results in the present study are consistent with results shown in Chapter 3 which examined the effects of an earlier model of the SmartStretch™ technology and ageing on tenderness. In Chapter 3 it was shown that the SmartStretch™ treatment led to a 46% reduction in shear force at 0 days ageing and after 5 days of ageing a 38% a reduction in shear force was still achieved. In the current study the SmartStretch™ treatment resulted in a 37% reduction in shear force at 0 days ageing and after 5 days of ageing in a 16% reduction in shear force. The interaction between SmartStretch™ treatment and ageing period was mostly consistent with the results in Chapter 3 (*M. semimembranosus*) for shear force which showed that the un-aged control group (no SmartStretch™) meat was the toughest and samples from the SmartStretch™ and 5 days ageing treatment were the most tender. However it should be noted that there was no significant difference between 0 day SmartStretch™ treatment and 5 day aged control treatment in the present study. As previously outlined in both Chapters 3 and 4 these benefits are supported by previous studies which excised and aimed to constrict *pre-rigor* muscle contraction through wrapping (Devine et al., 1999; Hildrum et al., 2000; Rosenvold et al., 2008).

Results from Chapters 3 and Devine et al. (1999) all indicate that by restricting the muscle from contracting one can reduce the variation in meat tenderness hence producing a more consistent product. However, the results in the current study are contrasting with the SmartStretch™ 5 day aged and stimulated treatment samples exhibiting the least variation and the SmartStretch™ treatment 0 day aged, and non

stimulated samples having the greatest variation. It is unclear why this has occurred, however overall SmartStretch™ did reduce the variation in shear force when stimulation and ageing treatments were removed from the model.

Results presented in Chapter 3 showed the SmartStretch™ treatment increased sarcomere length from 1.54 to 2.19 μm when compared to the control and this is consistent with the present study where irrespective of stimulation treatment the SmartStretch™ treatment increased sarcomere length from 1.53 to 2.27 μm . Based on these results and the results in Chapter 4 it can be concluded that SmartStretch™ successfully prevents muscle contracture and stretches sarcomeres in hot-boned sheep meat. It can also be confirmed that the SmartStretch™ treatment does increase the variability in sarcomere length (Chapters 3 and 4) in hot-boned sheep meat. This increased variability could be attributed to the varying degree of success that the SmartStretch™ treatment has on either reducing muscle contraction or stretching the muscle as opposed to the control treatment where muscle is able to contract.

Brewer (2004) defined water holding capacity as ‘the ability of meat to hold it’s own or added water when force is applied’. The majority of water in muscle is held either within the myofibrils or between myofibrils, between the myofibrils and cell membrane, between muscle cells and between muscle bundles (Huff-Lonergan & Lonergan, 2004). It has been shown that the amount and location of water can change once the muscle is harvested (Honikel, 2004). Honikel et al. (1986) concluded that the release of drip from muscles appeared to be dependent on the state of contraction (contracted sarcomeres, fibrils or fibres) after the onset of *rigor*. It was thought that this was due to the shrinkage of filament spacing which results in the release of water from cells (Honikel et al., 1986).

The effect that SmartStretch™ has on water holding capacity was examined in Chapter 4 by the assessment of purge and cooking loss. This assessment came about after there was some anecdotal evidence in Chapter 3 that although the control had significantly greater cooking loss in the *M. semimembranosus* there did appear to be more purge from SmartStretch™ samples. There was an inconsistent cooking loss result in Chapter 4 between the two muscles assessed (*Mm. biceps femoris* and *semimembranosus*). SmartStretch™ caused a reduction in cooking loss for the *M. biceps femoris* which was consistent with results in Chapter 3 and no change for the *M. semimembranosus*. It was hypothesised that this was because the *M. semimembranosus* was not stretched to the same extent in the whole leg scenario. This theory was further supported in the current study given the significant degree of stretch shown in the *M. semimembranosus* and the fact that SmartStretch™ also reduced the amount of cooking loss irrespective of stimulation treatment. However as eluded to in Chapter 3, SmartStretch™ also increased the amount of purge lost irrespective of stimulation treatment in the current study. This also supports the theory in Chapter 4 that SmartStretch™ may not impact on the overall water holding capacity of sheep meat when compared to control. Based on these conclusions the question is how does this outcome impact on the consumer? Both purge (Payne, Durham, Scott, & Devine, 1998) and cooking loss (Barbera & Tassone, 2006) can be viewed negatively by the consumer, but the former will be more important as it can be evidenced at the time of purchase.

Given consumers' decision to purchase meat is influenced by meat colour more than any other trait (Mancini & Hunt, 2005) it is considered to be one of the most important meat quality traits. It has been concluded that post slaughter factors, such as electrical stimulation, can impact meat colour (Devine et al., 2004). Results from the

current study indicate that medium voltage stimulation had no detrimental effect on retail colour display which is supported by results presented by Pearce et al. (2009). Thus it can be concluded in the present study that the stimulation treatment would not impact on consumer perceptions of colour in their decision to purchase.

From an economic perspective the colour stability of meat is critical. Jacob et al. (2007) reported that meat on retail display will begin to brown within 1-7 days. This browning occurs due to the formation of metmyoglobin which can be measured by the ratio of reflectance at 630 and 580 nm (Hunt, 1980). Stretch treatment had a positive effect on the colour stability based on ratio 630/580nm and a* values at both 0 and 5 days aged. The ratio 630/580nm values indicated that the formation metmyoglobin (browning of meat) occurred at a slower rate in samples subjected to the stretch treatment when compared to control. In addition the SmartStretch™ treatment a* values also decreased at a slower rate over time meaning that these samples maintained a red colour for longer. It is unclear how SmartStretch™ treatment has improved colour stability of the *M. semimembranosus*. However, it could be hypothesised that the SmartStretch™ treatment caused a reduction in oxygen consumption. This may be a result of less oxygen been able to penetrate the muscle due to the changes in structure and shape of the muscle and hence a slower formation of metmyoglobin and increased stability of the red colour, but, this is yet to be quantified. Stretch treatment had a minimal effect that on L* and b* meat colour values which is supported by a recent study by Bayraktaroglu & Kahraman, (2011) where it was reported that stretching beef *biceps femoris* had no significant effect on meat colour.

In both Chapters 3 and 4 it was shown that the SmartStretch™ treatment was successful in transforming the *M. semimembranosus* and whole tunnel boned sheep

legs into a consistent shaped product. In the present study on average there was a 30% increase in *M. semimembranosus* length and a 50% decrease in circumference at the first measurement site and 19% decrease in circumference at the second measurement. This consistency is considered a desirable trait by the food service industry (Anonymous, 2003) as it standardises shape for the preparation of roast and slices and minimises cutting losses.

5.5 Conclusions

Medium voltage stimulation did not impact on any of the meat quality traits tested, except reducing initial pH. Meat tenderness of the *M. semimembranosus* was improved significantly after 0 and 5 days of ageing by applying SmartStretch™ and sarcomere length was also increased. The improvements in tenderness as a result of the *pre-rigor* stretching device could remove the need for aged chiller storage to achieve acceptable tenderness levels through an accelerated processing system such as hot boning. Based on the ratio 630/580nm and a* colour values the stretch treatment could increase meat display time. Overall, the results from this study have highlighted significant potential for using the stretching device to improve sheep meat quality and consumer satisfaction and would suggest that in this commercial scenario medium voltage stimulation did not appear to inhibit the effectiveness of SmartStretch™ given on average muscles were still in a *pre-rigor* state.

Chapter 6: Improving the tenderness of beef topsides (*M. semimembranosus*) and Rostbiffs (*M. gluteus medius*) using a meat stretching device

6.1. Introduction

The challenge the meat industry faces in the quest to increase processing efficiency is to maintain or enhance eating quality and this is particularly relevant to the use of hot boning. As outlined in Chapter 3, despite the many advantages of hot boning for example efficient processing (Jeremiah, Martin, & Murray, 1985; Pisula & Tyburcy, 1996), there is a risk of comprising meat quality (Sponcer, 1993; Devine et al. 2004; Tornberg, 1996) especially with regard to muscle shortening due to the removal of the skeletal restraint during the hot boning process. For this reason hot boning is often performed with the use of electrical stimulation, which would accelerate glycolysis so that the muscles have passed through *rigor* prior to hot boning. An alternative approach to prevent muscle shortening is to restrain the muscles physically until they are in *rigor mortis* (Macfarlane, Harris, & Shorthose, 1974).

Sarcomere length was significantly increased in *M. semimembranosus* (Chapter 3; Chapter 4; Chapter5) and *M. semitendinosus* (Chapter 4). Based on the shear force results shown in Chapter 3 and 5 it was concluded that the stretching prototype device (licensed as SmartStretch™) significantly improved meat tenderness in sheep *M. semimembranosus* irrespective of stimulation treatment (Chapter 5). These tenderness improvements were also shown in Chapter 4 when a whole sheep leg was tested using the SmartStretch™ machine and shear force was tested on both the *Mm. semimembranosus* and *biceps femoris*.

However, preliminary studies on beef by Toohey, Kerr, van de Ven, & Hopkins (2010) and Taylor, Hopkins, & van de Ven (2010) using the same stretching prototype device (SmartStretch™) have shown contrasting results. An initial study by Toohey et al. (2010) evaluated the effect of three levels of stretch created by SmartStretch™ technology and ageing, on the tenderness of hot-boned beef *M. semimembranosus* taken from older cows with a dentition greater than two. Although significant changes in *M. semimembranosus* dimensions were achieved (52, 41 and 34% increase in muscle length) there was no significant tenderness or sarcomere length benefit from increasing the degree of stretch (Toohey et al., 2010).

Hwang, Gee, Polkinghorne, & Thompson (2002) showed that for the *M. semimembranosus* there is a threshold sarcomere length beyond which further stretching will not further reduce shear force. This is supported by Simmons et al. (1999) who showed that stretching beef *M. longissimus* 20% using clamps significantly reduced shear force, but that there were no additional benefits when muscles were stretched beyond this. Based on the percentage increase in muscle length shown by Toohey et al. (2010) it was concluded that the stretching threshold was achieved using SmartStretch™ with the lowest degree of stretch treatment. Based on previous sheep results (see Chapter 3; Chapter 4; Chapter 5), it was suggested that using the SmartStretch™ technology a minimum stretch of 30% in muscle length would produce a significant improvement in tenderness of hot boned beef *M. semimembranosus* compared to control, but this remains to be validated.

The later study by Taylor et al. (2010) examined the effect on objective meat tenderness and consumer acceptance of hot-boned *M. semimembranosus* and Rostbiffs (mainly the *M. gluteus medius*) taken from six aged cows. Results showed on average a 18.7N drop in *M. gluteus medius* shear force as a result of

SmartStretch™ technology, however this was not significant, suggesting that a larger sample size might have delivered a significant result.

Based on these previous studies it was still unclear if SmartStretch™ technology would be effective in improving beef meat quality in either *Mm. semimembranosus* or *gluteus medius*. In order to determine if SmartStretch™ technology can improve the tenderness of hot-boned beef, two experiments were designed. Experiment 1 used samples from cull cows to evaluate the effect of both stretch (SmartStretch™ and control) and ageing (0 and 14 days) on objective meat tenderness of hot-boned beef *M. semimembranosus*. The second experiment used samples from younger cattle to further validate using a larger sample size the effect of SmartStretch™ and ageing (0 and 8 days) on objective meat tenderness of hot-boned beef *M. gluteus medius*.

6.2 Materials and Methods

6.2.1. Experiment 1: Effect of stretch and ageing on hot-boned beef

***M. semimembranosus* meat quality.**

6.2.1.1 Animals

For testing the effect of the SmartStretch™ prototype machine and *post-mortem* ageing on beef *M. semimembranosus*, a total of 32 cow carcasses were randomly selected from various consignments killed over one day. The cows used from these different consignments were of varying backgrounds and were randomly assigned to treatment groups. The cows all had more than two and less than 8 permanent incisors

6.2.1.2 Experimental Design

The experiment was designed as a hybrid of a row/column and a split plot experiment. The rows of the design correspond to carcasses and the columns to the four cuts within each carcass. These four cuts within a carcass correspond to the left and right side of a carcass and the two ends (cranial and caudal) within each side. The four treatment combinations (two levels of stretch \times two levels of ageing) were allocated so that the two levels of stretch were assigned to the two sides of each carcass and the two levels of ageing to the two ends within each half. The design was balanced in that each ageing level occurred equally often at each end within each half of a carcass given each stretch treatment. Also, each stretch treatment level occurred equally often on each side of a carcass. The treatment combinations used were: 0 days ageing + SmartStretch™, 0 days ageing + control, 14 days ageing + SmartStretch™ and 14 days ageing + control. The treatments were rotated across position and ageing treatments.

6.2.1.3 Sample collection

The carcasses were processed under the normal commercial procedures of the abattoir. As part of this process animals were stunned (50 Hz, 70 volts, and a maximum current of 100mA, pulse width of 5-10 microseconds) followed by exsanguination. A further electrical input at immobilisation (50-60 Hz, 240 peak volts, a current of 1-2 amps, pulse width 150 microseconds) was applied for 45 secs. Both the right and left topsides (*Mm. semimembranosus*, *adductor* & *gracilis*) HAM. 2000 (Anonymous, 2005) from 32 carcasses ($n = 64$) were collected within 2 hours of stunning. The *M. semimembranosus* was removed from the topside (HAM 2000) and irrespective of stretch treatment samples were cut to similar dimensions with an

average circumference of 30 cm. The *M. semimembranosus* allocated to the SmartStretch™ treatment were ejected from the SmartStretch™ machine into the packaging unit with a circumference of 24 cm. Then each sample (stretch and control) was portioned into two sub-samples (cranial and caudal) which were allocated using a predetermined random allocation design to ageing treatments of 0 or 14 days. Samples were processed into their predetermined treatments within 2 hours of death.

6.2.1.4 Muscle measurements and initial pH and temperature

The initial muscle measurements were recorded immediately after muscle collection and were taken and conducted as described in section 3.2.4, and the final muscle measurements were recorded immediately after stretch treatment. The initial and final circumference was measured at three points along the muscle. Initial pH and temperature were measured prior to the application of the stretch treatment using the same method as described in section 3.2.5.

6.2.1.5 Treatments

The stretch treatment was applied within 2 hours of exsanguination. The treatment was achieved using a meat stretching prototype (SmartStretch™) as previously described in section 3.2.6.

The 0 day samples were placed in a freezer within 4 hours of slaughter and stored in a freezer at -22°C until sampling. The 14 day aged samples were chilled at an average temperature of 3-4°C and following 14 days of ageing were frozen and stored in a -22°C freezer.

6.2.1.6 Sampling procedures

The 0 day aged samples for shear force testing, cooking loss, thaw loss, final pH and sarcomere length were cut from frozen using a bandsaw.

The 14 day aged samples were held at 3-4°C and then purge was measured on the whole cut and shear force, cooking loss, thaw loss, final pH and sarcomere length samples were also cut to size and frozen and stored in a -22°C freezer until ready for further testing.

6.2.1.7 Thaw loss percentage

Thaw loss percent was calculated on 0 day aged samples by cutting a portion of meat to the following dimensions 4 x 4 x 5cm. An initial weight was recorded samples were then placed in a chiller on a rack with a drip tray placed below and the upper surface of the samples were covered in foil. Samples were held in the chiller at 3-4°C for 48 hours, whereupon they were removed from the chiller and placed on paper towelling where they were then patted dry and a final weight recorded. The thaw loss percentage was calculated using the following formula;

$$\text{Thaw loss (\%)} = 100 - (\text{Final weight/Initial weight} * 100)$$

6.2.1.8 Final pH, Warner Bratzler shear force, cooking loss percent and purge percentage loss

These measurements were taken and conducted as described in sections 4.2.9, 3.2.9, 3.2.10, 5.2.6 and 5.2.7 respectively. It should be noted that there were two 0 day control and six 0 day stretch treatment samples that could not be tested for shear force or cooking loss due to lack of sample.

6.2.1.9 Sarcomere length

Sarcomere length was measured initially on 0 day aged samples using laser diffraction as described by Bouton et al. (1978). From these samples double banding was observed on a proportion of the SmartStretch™ treatment samples only. Given the unusual result the laser diffraction unit was recalibrated and both 0 and 14 day aged samples were examined using a different operator. Results again indicated double banding in SmartStretch™ treatment samples only, in both 0 and 14 day aged samples. The bands showed one short band (associated with long sarcomeres) and a long band (associated with short sarcomeres). Five replicates of each sample were recorded. For samples that exhibited the double banding, two values were recorded and the mean of the five replicates was calculated for each band.

6.2.1.10 Statistical analysis

Linear mixed model analysis was undertaken using ASReml, (Gilmour et al. 2006) via the statistical package *asreml* (Butler, 2009) under R (R Development Core Team, 2009). The model fitted for each of the variables initial pH, initial temperature, purge percent, thaw loss percent, sarcomere length, shear force and cooking loss percent is shown in Table 6.1. Shear force samples were transformed to a log scale and the final cook loss percent model excluded the carcass x stretch treatment, cooking date and cook batch as sources of variance as they contributed little variation.

Table 6.1. The model structure for analysis of initial pH (ipH), initial temperature (itemp), final pH (fpH), shear force (SF), cooking loss (CL), purge loss, thaw loss, hot carcass weight (Cwgt) and fat depth (P8) using ASReml, showing the effects fitted as fixed (F) or random(R).

Variable	ipH	itemp	fpH	SF	Sarc	CL	Purge	Thaw	Cwgt	P8
Constant	F	F	F	F	F	F	F	F	F	F
Stretch	F	F	F	F	F	F	F	F	F	F
Age	-	-	F	F	F	F	-	-	-	-
Stretch x Age	-	-	F	F	F	F	-	-	-	-
Carcass	R	R	R	R	R	R	R	R	-	-
Carcass x Stretch	-	-	R	R	-	R	-	-	-	-
Portion	-	-	-	R	-	-	-	-	-	-
Cook date	-	-	-	R	-	R	-	-	-	-
Cook batch	-	-	-	R	-	R	-	-	-	-
Stretch x No bands	-	-	-	-	F	-	-	-	-	-
Stretch x No bands x Band Number	-	-	-	-	F	-	-	-	-	-
Stretch x Age x No bands x Band Number	-	-	-	-	F	-	-	-	-	-
Stretch x Age x No bands	-	-	-	-	F	-	-	-	-	-
Error	R	R	R	R	R	R	R	R	R	R

Further analysis was conducted to estimate the mean sarcomere length for each stretch treatment for samples having either one or two bands. In order to achieve this, estimates of the following model parameters were required.

μ_c = mean sarcomere length for control samples

μ_{s1} = mean sarcomere length for stretched samples with a single band

μ_{s21} = mean sarcomere length based on 1st band for stretched samples with two bands

μ_{s22} = mean sarcomere length based on 2nd band for stretched samples with two bands

p = proportion of stretched samples having two bands

To jointly estimate the parameters p , μ_c , μ_{s1} , μ_{s21} and μ_{s22} a Bayesian modelling approach was used. Basically the same model was fitted to the data as for linear mixed model analysis as shown in Table 6.1, but with the Age effect excluded as it was not significant. The model also included the parameter p . Very disperse priori are placed on the unknown parameters for the model. The model was fitted using the R package R2WinBUGS (Sturtz, Ligges, & Gelman, 2005) in R (R Development Core Team, 2010), with R2WinBUGS in turn using WinBugs (Spiegelhalter, Thomas & Best, 1999). Three chains were generated, each of length 1.05×10^6 , of which the first 50000 samples were discarded and then only 100th subsequent sample saved. Based on the simulated parameter values, including p , estimates of the combinations of parameters and associated standard errors are derived.

6.2.2. Experiment 2: Effect of stretch and ageing on hot-boned beef

***M. gluteus medius* meat quality.**

6.2.2.1 Animals

For testing the effect of the SmartStretchTM prototype machine and *post-mortem* ageing on beef *M. gluteus medius*, a total of 40 mixed sexed carcasses (female or castrate male) were randomly selected from various consignments killed over one

day. The carcasses used from these different consignments were of varying backgrounds and were randomly assigned to treatment groups. All carcasses had two or less permanent incisors

6.2.2.2 Experimental Design

The experiment was designed using the split plot design as described in Section 6.2.1.2. The treatment combinations used were; 0 days ageing + SmartStretch™, 0 days ageing + control, 8 days ageing + SmartStretch™ and 8 days ageing + control. There were two stages to the design for this experiment. The treatment combinations were randomised between carcass and side within carcass. Ageing treatment was applied for 8 days in this experiment due to logistical reasons.

6.2.2.3 Sample collection

The carcasses were processed under the normal commercial procedures of the abattoir. As a part of this process animals were slaughtered using captive bolt then carcasses were exposed to the electrical inputs routinely used by the cooperating abattoir including; a high frequency immobilisation unit (2000Hz, current of 300 milliamps, pulse width 100 microseconds) applied for 10-15secs and rigidity probe (180 volts rms, 50Hz, current of 300 milliamps, pulse width 1000 microseconds) applied for 7-10secs. Both the right and left Rostbiffs (mainly *M. gluteus medius*) HAM. 2110 (Anonymous 2005) from 40 carcasses ($n = 80$) were collected and then portioned into ageing treatments ($n = 160$). Irrespective of stretch treatment samples were cut to similar dimensions with an average circumference of approximately 30 cm. Samples were then allocated to allocated treatments as described in section 6.2.1.3.

6.2.1.4 Muscle measurements, initial pH and temperature, treatments, sampling procedures, Warner Bratzler shear force, final pH, sarcomere length, cooking loss percent and purge percentage loss.

These measurements were taken and conducted as described in sections 3.2.4, 3.2.5, 3.2.6, 6.2.1.6, 3.2.9, 4.2.9, 3.2.8, 3.2.10, and 5.2.7 respectively. The initial and final circumference was measured at three points along the muscle. The 0 and 8 day ageing treatments were also applied as described in section 3.2.6. Purge loss was conducted on 8 day aged samples only. Sarcomere length was measured on 0 day aged samples only.

6.2.2.5 Statistical analysis

Linear mixed model analysis was undertaken using ASReml, (Gilmour et al. 2006) via the statistical package *asreml* (Butler, 2009) under R (R Development Core Team, 2009) were used to analyse the data. The models fitted for initial pH, initial temperature, purge percent and sarcomere length, cooking loss percent and shear force are shown in Table 6.2

Table 6.2. The model structure for analysis of initial pH (ipH), initial temperature (itemp), final pH (fpH), sarcomere length (Sarc), shear force (SF), cooking loss (CL), purge loss, hot carcass weight (Cwgt) and fat depth (P8) using ASReml, showing the effects fitted as fixed (F) or random (R).

Variable	ipH/ temp	fpH	SF	Sarc	CL	Purge	Cwgt/P8
Constant	F	F	F	F	F	F	F
Stretch	F	F	F	F	F	F	F
Age	-	F	F	-	F	-	-
Stretch x Age	-	F	F	-	F	-	-
Carcass	R	R	R	R	R	R	-
Carcass x Side	-	R	R	-	R	-	-
Initial pH	-	-	F	-	R	-	-
Stretch x Initial pH	-	-	F	-	R	-	-
Initial pH x Age	-	-	F	-	-	-	-
Stretch x Initial pH x Age	-	-	F	-	-	-	-
Cook batch	-	-	R	-	R	-	-
Error	R	R	R	R	R	R	R

6.3 Results

6.3.1 Muscle measurements

The results shown in Table 6.3 indicate that the SmartStretch™ treatment greatly altered the pre stretch treatment shape in both experiments 1 and 2 by increasing the length and decreasing the circumference when measured at three different points along the both the *Mm. semimembranosus and gluteus medius*.

Table 6.3. Raw means, standard error (s.e.) and range for experiment 1 *M. semimembranosus* (SM) and experiment 2 *M. gluteus medius* (GM) % increase in length and % decrease in circumference for SmartStretch™ treatment.

Trait	Experiment 1 (SM)		Experiment 2 (GM)	
	Mean (s.e.)	Range	Mean (s.e.)	Range
Increase length (%)	40.5(2.09)	21.0-66.7	32.3 (2.46)	4.9-50.0
Decrease circumference 1 (%)	25.4(0.87)	4.7-31.9	34.5 (0.67)	21.4-43.4
Decrease circumference 2 (%)	24.8 (0.73)	8.7-31.3	34.9 (0.61)	28.9-46.4
Decrease circumference 3 (%)	28.1 (0.77)	21.7-38.6	34.0 (2.08)	13.2-100.0

The predicted mean, standard error and range, for hot carcass weight, P8, initial pH, initial temperature and final pH are shown in Table 6.4. There was no significant difference ($P > 0.05$) between stretch and ageing treatments for any of the traits except for experiment 1 final pH as shown in Table 6.4 nor were there any interactions. This indicates that the random allocation of muscles across treatment groups was balanced. There was a significant ageing effect ($P = 0.02$) in experiment 1 for final pH such that the 14 day aged control treatment had a significantly higher pH (5.62) when compared to 0 day control (5.57) and SmartStretch™ (5.58). However the 14 day aged SmartStretch™ samples were not statistically different ($P > 0.05$) to any other treatment samples (5.59).

Table 6.4. Predicted means, standard error (s.e.) and range for experiment 1 *M. semimembranosus* (SM) and experiment 2 *M. gluteus medius* (GM) hot carcass weight, P8, initial pH, initial temperature, Final pH, for SmartStretch™ and control treatment.

Trait	Experiment 1 (SM)			Experiment 2 (GM)		
	Control	SmartStretch™	Control	SmartStretch™	Control	SmartStretch™
	Mean (s.e.)	Range	Mean (s.e.)	Range	Mean (s.e.)	Range
Hot carcass weight (side) (kg)	205.2 (8.1)	128.5-311.0	205.2 (8.1)	128.5-311.0	135.4 (3.30)	103.5-189.9
P8 (mm)	5.0 (0.92)	1.0-20.0	5.0 (0.92)	1.0-20.0	9.6 (0.53)	5.0-20.0
Initial pH	6.13 (0.04)	5.58-6.66	6.10 (0.04)	5.68-6.58	6.06 (0.02)	5.82-6.44
Initial temperature (°C)	36.9(0.33)	33.6-40.3	36.6(0.33)	33.9-39.9	39.8 (0.10)	38.4-41.0
Final pH – 0 day	5.57(0.02)	5.43-6.05	5.58(0.03)	5.35-6.15	5.55 (0.02)	5.43-5.82
- 14 days	5.62(0.02)	5.47-5.97	5.59(0.02)	5.40-5.84	5.56 (0.01)	5.48-5.81
- 8 days					5.54 (0.01)	5.48-5.68

6.3.2 Warner Bratzler Shear force

Results from experiment 1 show that there was a significant effect on shear force between ageing treatments ($P < 0.001$) such that as ageing time increased, meat tenderness also increased 0 day ($65\text{N} \pm 2.4$) versus 14 day aged ($48\text{N} \pm 1.7$). When initial pH was added as a covariate there was no impact on shear force ($P = 0.34$) for stretch treatment. Of the random terms fitted to the model, carcass and cooking batch were significant ($P < 0.05$). It is unclear why cook batch was significant, but the results indicate that samples cooked on the second day were consistently lower in shear force. It is not unexpected that there was carcass variance given the range of animals sampled however this variance could indicate that some muscles may not have responded as well to the SmartStretch™ treatment.

Contrasting results were shown in experiment 2 where there was a significant effect on shear force for the SmartStretch™ treatment ($P < 0.001$), ageing treatment ($P < 0.001$) and the interaction between both of these treatments ($P < 0.05$) as shown in Table 6.5.

Table 6.5. Wald-statistic F -ratios for effects stretch, age, stretch x age along with relevant covariate for initial pH on shear force experiment 1 *M. semimembranosus* (SM), experiment 2 *M. gluteus medius* (GM).

Terms	Shear force (N)			
	Experiment 1 (SM)		Experiment 2 (GM)	
	df	F -ratio	df	F -ratio
Stretch	1,60	0.00 ^{ns}	^	^
Age	1,60	88.11***	^	^
Age x Stretch	1,60	2.32 ^{ns}	1,113	11.88**
Initial pH	1,59	0.92 ^{ns}	1,91	3.87 ^{ns}

*** $P < 0.001$; ** $P < 0.01$; ^{ns} $P > 0.05$; ^ Not tested because there was a significant interaction.

The significant interaction ($P < 0.05$) between SmartStretch™ treatment and ageing period showed that the 0 day aged control group (no SmartStretch™) meat was the toughest and the benefits of the stretching treatment diminished after 8 days of

ageing (Table 6.6). When initial pH was used as a covariate for shear force it showed that the regression was only significant at 0 day (*Wald Statistic: $F(1,113)=4.84$ $P=0.03$*) such that as initial pH increased so did the shear force values at 0 day aged irrespective of treatment.

Table 6.6. Predicted shear force means (N) and standard errors (s.e.) for each stretch and ageing treatment for experiment 2 *M. gluteus medius* (GM).

Treatments	Experiment 2 (GM)	
	0 day	8 day
Control	66.4 (1.84) c	35.8 (1.83) a
SmartStretch™	53.3 (1.83) b	33.6 (1.83) a

Means followed by a different letter within experiment (a, b, c) are significantly different $P = 0.05$.

Results from both experiment 1 and 2 indicate that stretch treatment had no impact on the variation in shear force, but greater variation was shown in 0 day aged samples compared to samples aged for either 8 or 14 days.

6.3.3 Cooking loss

In both experiments 1 and 2 there was a significant interaction between stretch and ageing treatments for cooking loss (*Wald Statistic: $F(1,58)=11.88$ $P=0.001$*) and (*Wald Statistic: $F(1,78)=4.66$ $P=0.03$*) respectively. Although in experiment 1 the cooking loss was slightly higher for the 0 day control it was not significant ($P > 0.05$), however 0 day control samples in experiment 2 did have a significantly higher ($P < 0.05$) cook loss as shown in Table 6.7. After 14 days of ageing the stretch treatment samples in experiment 1 had significantly ($P < 0.05$) greater cooking, but after 8 days of ageing in experiment 2 there was no difference ($P > 0.05$) between treatments. For both experiments 1 and 2, irrespective of stretch treatment cooking loss was significantly greater ($P < 0.05$) for 14 and 8 day aged samples when compared to 0 day aged samples.

Table 6.7. Predicted cooking loss means (%) and standard errors (s.e.) for each stretch and ageing treatment for experiment 1 *M. semimembranosus* (SM) and 2 *M. gluteus medius* (GM).

Treatments	Experiment 1 (SM)		Experiment 2 (GM)	
	0 day	14 day	0 day	8 day
Control	21.7 (0.54) a	23.6 (0.53) b	21.0 (0.40) b	23.2 (0.40) c
SmartStretch™	20.8 (0.58) a	25.9 (0.52) c	19.6 (0.40) a	23.1 (0.40) c

Means followed by a different letter within experiment (a, b, c) are significantly different $P = 0.05$.

6.3.4 Purge and thaw loss percentage

In experiment 1 there was no significant difference (Table 6.8) between stretch and control treatments for purge loss examined at 14 days (*Wald Statistic: $F(1,31)=0.14$ $P=0.71$*) respectively. Similar results were also shown in experiment 2 when purge was examined after 8 days ageing (*Wald Statistic: $F(1,74)=2.62$ $P=0.11$*). SmartStretch™ treatment did result however in a significant ($P < 0.05$) reduction in thaw loss (Table 6.8) in *M. semimembranosus* aged for 0 days (experiment 1), (*Wald Statistic: $F(1,31)=8.52$ $P=0.006$*).

Table 6.8. Predicted means and standard errors (s.e.) for each stretch treatment for experiment 1 purge and thaw loss (%) and experiment 2 purge loss (%).

	Control	SmartStretch™
Experiment 1 Purge loss (14 day) (%)	2.61 (0.07)a	2.96 (0.25)a
Experiment 2 Purge loss (8 day) (%)	2.54 (0.14)a	2.23 (0.14)a
Experiment 1 Thaw loss (0 day) (%)	11.3 (0.55)b	9.7 (0.55)a

Means followed by a different letter within row (a, b) are significantly different $P = 0.05$.

6.3.5 Sarcomere Length

In experiment 1 a proportion of 0 and 14 day aged SmartStretch™ treatment samples exhibited double banding of the sarcomeres when measured using laser diffraction, hence two sarcomere values were recorded. This banding was not observed in any of the control treatment samples. Table 6.9 gives a breakdown on the

number of samples tested for each treatment and the number that displayed the double banding.

Table 6.9. Proportion of samples with single and double bands according to stretch and ageing treatments.

Treatment	0 Day aged	14 Day aged
Control	31	30
SmartStretch™ single band	8	11
SmartStretch™ double band	23	20

A graphical summary of the data collected is shown in Figure 6.1, wherein the box-plots illustrate the distribution of the mean sarcomere lengths across treatments (Mean SL), where each mean is the average of five replicates. The box-plots are given separately for Band 1 when there is only one band visible and for both bands when two bands are visible.

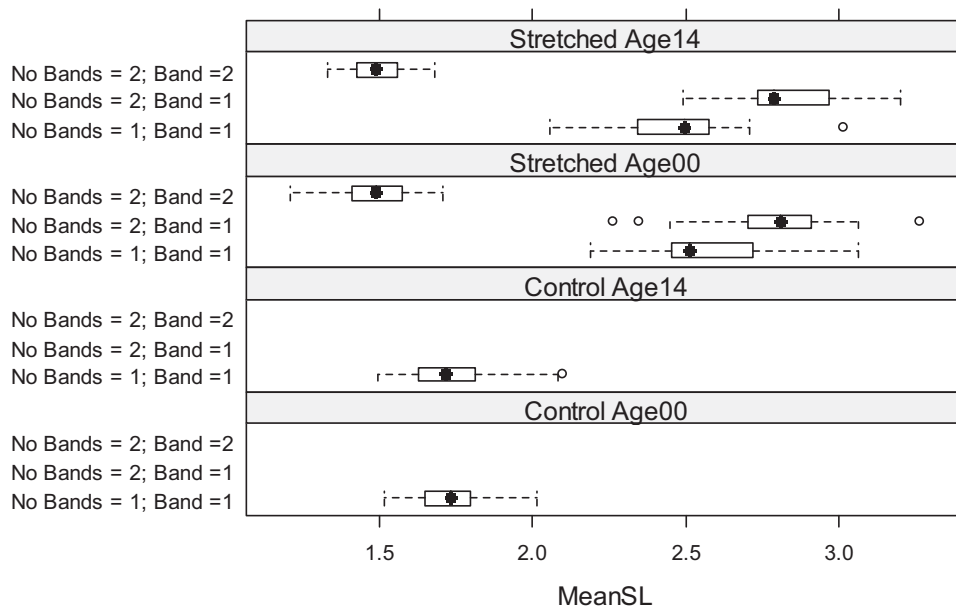


Figure 6.1. Mean sarcomere lengths across treatments (Mean SL; μm) according to the number of bands within each treatment combination.

An analysis for significant differences across these eight separate combinations, indicates that there was no significant ageing effect across or within any of the other

treatment combinations (*Wald Statistic: $F(4, 107) = 0.842, P = 0.50$*). In addition a chi-squared test performed to examine if there was a significant difference in the proportion of samples within the two age groups having 2 bands indicated that there was no significant difference ($X^2 = 0.304, P = 0.58$). Hence the predicted means for sarcomere length for each stretch treatment and band, averaged across ages, were obtained and these, together with standard errors and an LSD ranking, are given in Table 6.10. From this it is shown that the SmartStretch™ first band has a significantly longer mean sarcomere length and second band has a significantly shorter mean sarcomere length than control, SmartStretch™ single band and SmartStretch™ first band. When only a single band was recorded within each sample the SmartStretch™ treatment were significantly longer when compared to control treatment (*Wald Statistic: $F(1, 158) = 43.93, P < 0.001$*).

Table 6.10. Predicted sarcomere length means (μm) and standard errors (s.e.) for stretch treatment using a linear mixed model.

Treatments	Number of bands	Mean Sarcomere length μm (s.e.)
Control	single band	1.74 (0.03) b
SmartStretch™	single band	2.50 (0.04) c
SmartStretch™	double band 1 st	2.82 (0.03) d
SmartStretch™	double band 2 nd	1.49 (0.03) a

Means followed by a different letter (a, b, c, d) are significantly different $P = 0.05$.

Further analysis was conducted to estimate the mean sarcomere length for each stretch treatment, within or across samples having one or two bands using a Bayesian modelling approach. Based on the simulated posterior values for the parameters, Table 6.11 shows a summary of the parameter estimates, together with the associated standard errors.

Table 6.11. Predicted sarcomere length means (μm) and standard errors for stretch treatment using a Bayesian model where P is the proportion of stretched samples having two bands.

Parameter	Estimate (Standard error)
μ_c (Control samples)	1.74 (0.03)
μ_{s1} (Stretched samples, with 1 band)	2.50 (0.04)
μ_{s21} (1 st band, Stretched samples, with 2 bands)	2.82 (0.03)
μ_{s22} (2 nd band, Stretched samples, with 2 bands)	1.49 (0.03)
p (proportion of stretched samples having two bands)	0.687 (0.057)
Carcase variance	0.0101 (0.0035)
Error variance	0.0202 (0.0029)

From Table 6.11 the estimates of the means for each of the four groups and associated standard errors were in good agreement with the estimates based on linear mixed model analysis. Based on the simulated parameter values, including p , it is possible to estimate combinations of parameters and associated standard errors.

In contrast this double banding was not shown for any samples observed in experiment 2. The results show that there was a significant difference ($P < 0.05$) between SmartStretch™ treatment and control (*Wald Statistic: $F(1,78)=26.6$ $P=0.001$*) for sarcomere length where SmartStretch™ samples also had significantly longer sarcomeres $1.92 \mu\text{m}$ (0.03) compared to controls at $1.70 \mu\text{m}$ (0.03). Results indicate that the variation in sarcomere length for the SmartStretch™ treatment groups was greater than the control treatment groups in both experiments.

6.4 Discussion

Based on the initial pH and temperature of the *M. semimembranosus* in experiment 1 and *M. gluteus medius* in experiment 2, some individual muscles were still in the *pre-rigor* phase when the stretch treatment was applied with a mean pH of 6.12 and 6.08 respectively and a mean temperature of 37 and 40°C respectively. However given the range in initial pH it was concluded that some muscles would have entered stages of *rigor*. This does not appear to have significantly impacted the

effectiveness of the stretch treatment given the relationship between initial pH and both shear force. This relationship was not significant in experiment 1, however in experiment 2 it was shown that as initial pH increased so did the shear force values at 0 day aged irrespective of stretch treatment. It is not clear if this is a result of shortening, however based on the improvements achieved in sarcomere length as a result of SmartStretch™ treatment this seems unlikely. In addition the exact pH at time of freezing is unknown making it difficult to confirm if it is a result of any shortening.

SmartStretch™ treatment had no significant effect on the shear force of *M. semimembranosus* from aged cows after either 0 or 14 days ageing (experiment 1). This is in contrast with previous results where SmartStretch™ was found to significantly improve aged sheep *M. semimembranosus* shear force (Chapters 3-5). This result was not predicted given that on average a 40% increase in *M. semimembranosus* muscle length was achieved using SmartStretch™ and based on previous work it was concluded that 20% stretch caused significant reductions in the shear force of New Zealand prime beef *M. longissimus* (Simmons et al., 1999). However the *M. semimembranosus* results in the present study do support outcomes by Hildrum et al. (2000) where wrapped *M. semimembranosus* from young bulls showed no difference in shear force at either 2 or 7 days of ageing. This is based on the assumption that wrapping and stretching have the same effect.

A review paper by Sørheim & Hildrum (2002) hypothesised that given the size and shape of beef *M. semimembranosus* it is physically more difficult to reduce fibre contraction compared to other muscles such as the *M. longissimus*. This notion of size could provide some explanation as to why there was no significant affect on beef *M. semimembranosus* shear force in the current study (although the *M. semimembranosus*

was portioned), but there was in sheep *M. semimembranosus* (Chapters 3-5). In contrast, the results of experiment 2 showed that SmartStretch™ treatment did have a significant effect on beef *M. gluteus medius* shear force at 0 days ageing, but this effect was diminished after 8 days of ageing. This outcome supports previous findings on sheep *M. semimembranosus* when stretched as part of a whole leg (Chapter 4). These results have highlighted the potential tenderness benefits that can be achieved with the application of partial hot boning of a muscle from the hind leg. This outcome could assist in the adoption of this boning method in industry by not only providing an alternative to value adding to lower quality cuts, but also provide a solution to the heat toughening issue caused by rapid pH decline.

Overall based on the shear force results of both experiments 1 and 2 it suggests that there are other factors that impact on the effectiveness of the SmartStretch™ treatment. Connective tissue is one aspect that may explain the lack of difference shown between treatments for shear force in experiment 1 given these samples were taken from older animals as opposed to experiment 2 where the muscles were sampled were from younger cattle. It has been well established that as animals age the structural integrity of connective tissue increases (Shorthose & Harris, 1990) and these increases have been related to increases shear force (e.g. Parrish, Bailey, & Naumann, 1961; Purslow, 2005).

In a study by Buck & Black (1968) it was shown that stretched *M. longissimus* from cull dairy cows tended to show greater amounts of perimysial tissue denaturation in cooked meat when compared to non stretched muscles. It was concluded that the improvements in tenderness may be accounted for by the mechanical thinning of connective tissue due to stretching (Buck & Black, 1968). However in the study by Herring et al. (1967) where excised muscles were mechanically stretched using

clamps, the level of connective tissue was not shown to affect tenderness. It was hypothesised that connective tissue may not play a major role in meat tenderness in muscles that contain smaller amounts of connective tissue (Buck & Black, 1968). This notion may explain why only 20% stretch was required to significantly reduce shear force of beef *M. longissimus* (Simmons et al., 1999) and yet a 40% increase in *M. semimembranosus* length did not confer a decrease in shear force given the higher connective tissue content of *M. semimembranosus*. Clearly further investigation is required to gain a greater understanding of how the amount of connective tissue impacts on the effectiveness of the SmartStretch™ treatment.

Additionally, irrespective of days aged when the sarcomere lengths were assessed in experiment 1, a proportion of SmartStretch™ treatment sarcomere results exhibited potential contracture bands when measured via laser diffraction. This resulted in one short band (associated with long sarcomeres) and one long band (associated with short sarcomeres). Contracture bands have been shown to be associated with electrical stimulation (Hwang et al., 2003a), but have also been observed in pale soft exudative meat (Bendall & Wismer-Pendersen, 1962), thaw shortened (Stromer, Goll, & Roth, 1967), cold shortened (Marsh, Leet, & Dickson, 1974) and high temperature treated muscles (Fabiansson & Libelius, 1985). These contracture bands are presumably caused by localised excessive release of calcium ions from the sarcoplasmic reticulum through a tetanic contracture. The impact of contracture bands on meat tenderness is unclear given that the sarcomere structure is not uniform and in experiment 1 there was no difference shown in shear force. Based on these outcomes a closer examination of the impact of SmartStretch™ on muscle structure from older beef is required.

The impact of SmartStretch™ on indicators of water holding capacity (purge, thaw loss and cooking loss) appears to be minimal, which is overall consistent with previous work (Chapters 4-5). For example in experiment 1 SmartStretch™ treatment showed a greater cook loss at 14 days, but less thaw loss and purge and 0 day cook loss was unaffected.

SmartStretch™ technology has demonstrated the ability to successfully transform beef cuts. In experiment 1 average a 40% increase in *M. semimembranosus* muscle length and a 26% decrease in circumference averaged across 3 measurement sites was observed. Experiment 2 had similar results with an average increase in length of *M. gluteus medius* of 32% and a 34% decrease circumference averaged across 3 measurement sites. This outcome supports the findings shown in Chapters 3-5 where sheep *M. semimembranosus* and whole tunnel boned sheep legs were transformed into a consistent shaped product. These dimensional changes were also achieved in earlier beef work by both Toohey et al. (2010) and Taylor et al. (2010), indicating that SmartStretch™ is able to successfully transform both beef and sheep meat cuts into a consistent shape. This is a desirable trait by the food service industry (Anonymous 2003) as it standardises preparation of roast and slices and minimises cutting losses and satisfies consumer demand for a more uniform and portion controlled product (Tarrant, 1998).

6.5 Conclusions

Shear force results in experiment 1 indicated that the SmartStretch™ treatment was unable to significantly improve meat tenderness in aged cow *M. semimembranosus*, however benefits could be achieved when the same treatment was applied to *M. gluteus medius* in younger cattle as shown in experiment 2. This

outcome could be invaluable to industry if by removing some hindleg muscles from quality beef carcasses at risk of heat toughening due to rapid pH decline this could aid in the reduction in carcass/muscle temperature with the aim to avoid heat toughening. Based on the sarcomere results displayed for experiment 1 it is unclear whether the lack of difference in shear force could be due to potential contracture bands caused by the SmartStretch™ treatment despite overall sarcomere length being significantly higher or whether other factors such as connective tissue impeded the effectiveness of SmartStretch™ treatment. Thus it is concluded that to gain a greater understanding of the impact of SmartStretch™ on beef meat tenderness further investigation into muscle structure of treated samples is required.

Chapter 7: General discussion and conclusions

7.1 General Discussion

Hot boning involves the removal of the muscles from the carcass *pre-rigor* and has proven to be successful in reducing costs and increasing processing efficiency compared with conventional boning of chilled carcasses. However, hot boning is often associated with reduced tenderness and it has been established that without the use of additional processing interventions sheep meat processed through this system without extended ageing periods is unacceptable by Australian consumer standards (Toohey & Hopkins, 2006a).

As discussed in Chapter 2 there are many factors which contribute to meat quality traits including production factors (e.g. breed, growth path), pre-slaughter factors (e.g. handling, nutrition) and post-slaughter factors (e.g. electrical stimulation, chilling). Research has shown that despite the importance of all of these factors what happens post-slaughter can have the greatest impact on meat quality. Hot boning allows individual muscles to be manipulated according to their intrinsic properties during the critical early *post-mortem* period (White et al., 2006). Previous work has shown in principle that hot-boned primals (sheep and beef) can be manipulated by mechanical clamping (Herring et al., 1965) and wrapping in sheep and beef (Devine et al., 1999). The Pi-Vac Elasto Pack System® was developed for commercial use and has been designed around beef cuts (Troy, 2006). Unfortunately, there has been limited commercial adoption of any of these techniques. Unlike previous work this study was aimed at examining the impact of a new novel processing technique licensed as SmartStretch™ on meat quality attributes of both sheep and beef under commercial processing conditions. This technology was designed to not only stretch *pre-rigor* muscle, but also constrict the muscle.

An initial experiment (Chapter 3) was conducted to determine if this new technology could improve objective meat tenderness in sheep topsides (*Mm. semimembranosus*, *adductor* and *gracilis*). Results showed that meat tenderness of the *M. semimembranosus* was significantly improved with a 46% or 34N reduction in shear force after 0 days of ageing and 38% or 20N after 5 days of ageing. A subsequent study (Chapter 4) in sheep showed that SmartStretch™ technology could also improve tenderness on whole hot boned legs. In this case SmartStretch™ caused a 13% (5.5N) reduction in *M. biceps femoris* shear force irrespective of ageing treatment. The benefits were also shown in shear force results for the *M. semimembranosus* where SmartStretch™ caused a 15% (9.2N) reduction in shear force after 0 days of ageing. However, after 5 days of ageing the benefits of SmartStretch™ were nullified. It was also demonstrated that stretching of hot-boned sheep sub-primals, like the topside, could be achieved without the manipulation of existing electrical stimulation settings (Chapter 5). In this study a 37% (27.5N) and 16% (7.2N) reduction in *M. semimembranosus* shear force at 0 and 5 days of ageing respectively was achieved, irrespective of whether medium voltage electrical stimulation was applied.

Based on the sheep meat tenderness results presented in Chapters 3 to 5 it can be concluded that SmartStretch™ technology has the potential to significantly improve tenderness. The improvements in tenderness are likely to be related to the ability of the SmartStretch™ technology has to prevent sarcomere shortening in sheep meat as sarcomere length was significantly increased when compared to controls as reported in Chapters 3 to 5. Based on the particle size results (indication of myofibrillar degradation) presented in Chapters 3 and 4 and histology results in Chapter 4 there is no evidence that proteolysis was affected by the SmartStretch™ treatment.

There was however a poor predictive relationship between the increase in primal length due to stretching and measures of shear force or sarcomere length. This suggests that the benefits of the stretching were not solely attributable to changes in sarcomere length. This technology not only stretches whole muscles, but it compresses and constricts muscles as shown by the dimensional changes achieved by the SmartStretch™ treatment. Based on the work covered here, it is not yet fully understood what mechanisms are responsible for the reductions in shear force. Overall the results presented in Chapters 3 to 5 have shown that SmartStretch™ technology could be used by the industry to improve the tenderness of hot-boned hind leg sheep meat. The prevention of toughening during the *rigor* development could remove the need for chiller storage during ageing to achieve acceptable tenderness levels.

In addition to the benefits shown in tenderness it also appeared that the SmartStretch™ treatment would not have any detrimental effects on other key meat quality traits such as water holding capacity and meat colour. Initial results presented in Chapter 3 showed that SmartStretch™ decreased cooking loss which is consistent with early work by Bouton et al. (1973a) who concluded that stretching meat increased the water holding capacity. However observations of increased purge were confirmed in both Chapters 4 and 5. This increase in purge could be a result of muscle compression caused by the SmartStretch™ treatment. There did not appear to be any negative effects on fresh colour or colour stability of sheep meat by the application of SmartStretch™. Based on the ratio 630/580nm (indication of browning) and a* colour values stretch treatment could possibly increase meat display time.

Following the favourable results in sheep meat, the technology was further examined under commercial beef hot boning conditions. Initially cull cows were used to represent the class of cattle typically processed under these conditions. Based on a

series of experiments, including experiment 1 reported in Chapter 6 it was found that stretching hot-boned beef *M. semimembranosus* taken from cull cows had little impact on meat quality. However the SmartStretch™ technology was proven to successfully stretch hot-boned *M. semimembranosus* muscle length by on average 40%, but this did not translate into an improvement in tenderness. By contrast, a later study on younger cattle (experiment 2) with a dentition score of 2 or less showed that significant improvements in the tenderness of hot-boned *M. gluteus medius* could be achieved (Chapter 6), consistent with results presented in Chapters 3 to 5. In experiment 2 (Chapter 6), a 34% increase in muscle length resulted in a 20% (13.1N) reduction in shear force after 0 days of ageing. This positive impact on tenderness was nullified with ageing, but the tenderness improvement indicated that there is scope for stretching to replace costly chilled storage. Preliminary work indicated that there was an improvement in beef striploin tenderness with even less stretch (mean 16%), which nearly persisted after 14 days of ageing (Geesink & Thompson, 2008). These outcomes open the way for the adoption of partial hot boning of lower value cuts to enhance value. Additionally this process may have specific application to aid processing of carcasses that are at risk of heat toughening by the removal of muscles (particularly in the hind leg) and hence will reduce the heat load of the carcass and allow a more rapid chill.

The suggestion by Simmons et al. (1999) that a minimum stretch of 20% was required to detect a beneficial effect in tenderness was not supported by the current work which indicated that this requirement may vary according to animal type and primal. As found with sheep there is a poor predictive relationship between the increase in primal length due to stretching and measures of shear force or sarcomere length. This indicates that there are other factors driving the changes in shear force.

Despite the non linear relationship, sarcomere length has been shown to influence shear force below a threshold length. Results from experiment 1 in Chapter 6 showed that irrespective of days aged a proportion of SmartStretch™ treatment sarcomere results exhibited potential contracture bands when measured via laser diffraction. The impact of these contracture bands on meat tenderness is unclear given that the sarcomere structure was not uniform and the lack of difference shown in shear force.

It was also hypothesised based on the shear force results presented in Chapter 6 that connective tissue is one aspect that may explain the lack of difference shown between stretch treatments in experiment 1, given that these samples were taken from older animals and there is a strong association with increases in connective tissue strength and increasing animal age (Shorthose & Harris, 1990).

An additional theory to explain the inconsistent effect of SmartStretch™ technology on meat tenderness could be related to muscle volume. It was hypothesised by Sørheim & Hildrum (2002) that given the size and shape of beef *M. semimembranosus* it is physically more difficult to reduce fibre contraction compared to other muscles such as the *M. longissimus*. It is not clear whether the increase in volume when a whole sheep leg is stretched contributed to the diluted effect or whether this was more likely due to the difficulty of aligning the fibres of various primals tested. It is suspected that it is more likely the latter, but based on the beef results this is also worthy of further investigation with a proposed up scaled machine.

Despite the inconsistent results shown in beef tenderness the impact of SmartStretch™ on indicators of water holding capacity (purge, thaw loss and cooking loss) appears to be minimal. This is consistent overall with previous work (Chapters 4-5). Although the impact on beef meat colour was not presented in the current work,

previous preliminary studies have shown that there was no negative effect (Toohey et al., 2010; Taylor et al., 2010) which is also consistent with work shown in sheep (Chapter 5).

7.2 Implications for industry

This work has shown that it is possible to achieve tenderness benefits in hot-boned sheep meat and to a lesser extent in hot-boned beef meat by using SmartStretch™ technology. The application of this technology in the current sheep industry is limited given that most hot-boned meat is from aged sheep and is often exported to countries where there is no requirement for tenderness. However, this technology does provide a useful tool to increase processing efficiency through greater application of hot boning, value adding to poorer quality meat and could assist the creation of new markets. The outcomes shown in beef have demonstrated potential tenderness benefits that can be achieved with the application of partial hot boning of a muscle from the hind leg. This outcome could assist the adoption of this boning method in industry by not only providing an alternative to value adding lower quality cuts, but also provide a solution to the heat toughening issue caused by rapid pH decline. However based on results presented and other preliminary studies by Toohey et al. (2010) the application of the technology by beef processors who hot bone cull cows is likely to be limited apart from the ability that the technology confers for improving shape.

7.3. Future research

Based on the results presented the impact that SmartStretch™ technology has on connective tissue structure is unclear. It would be of interest to identify whether the contribution of connective tissue to toughness can be altered and/or if there is a threshold that connective reaches which can no longer be counteracted by SmartStretch™. Hence it would be of value to assess connective tissue in a range of SmartStretch™ treated muscles from a range of animal types. It would also be of interest to further investigate the occurrence of the potential contracture bands observed in Chapter 6, experiment 1 using an electron microscope to gain a greater understanding of the impact of SmartStretch™ technology on sarcomere structure. In addition, despite the benefits shown in objective meat tenderness it is not known whether this would transfer to greater consumer acceptance. If the technology is applied for stretching meat then more comprehensive research is required to establish the impact on eating quality and how this would relate to MSA standards.

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Appendix

Publications arising from research reported in this thesis

Conference proceedings

Toohey, E., Hopkins, D., & Lamb, T. A. (2008). The impact of wrapping and ageing hot boned sheep meat on eating quality. *Proceedings 54th International Congress of Meat Science and Technology*, Session 7B.19, Capetown, South Africa.

Toohey, E., Hopkins, D., Lamb, T. A., Nielsen, S. G., & Gutzke, D. (2008). Accelerated tenderness of sheep topsides using a meat stretching device. *Proceedings 54th International Congress of Meat Science and Technology*, Session 7B.18, Capetown, South Africa.

Toohey, E., Hopkins, D., Nielsen, S., & Gutzke, D. (2009). Impact of a meat stretching device on sheep meat quality. *Proceedings 55th International Congress of Meat Science and Technology*, Session, PE4.14, Copenhagen, Denmark.

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Toohey, E., Kerr, M. J., van de Ven, R., & Hopkins, D. (2010). The effect of Smart Stretch technology on the tenderness of beef cube roll. *Proceedings Australian Society of Animal Production*. (pp. 47), Armidale, Australia.

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Toohey, E., Kerr, M. J., van de Ven, R., & Hopkins, D. (2010). The effect of SmartStretch technology on the tenderness of beef topsides and cube rolls. *Proceedings 56th International Congress of Meat Science and Technology*, Session E072, Jeju, South Korea.

Scientific Journal

Toohey, E. S., van de Ven, R., Thompson, J. M., Geesink, G. H., & Hopkins, D. L. (2011). SmartStretch™ Technology. I. Improving the tenderness of sheep topsides (*M. semimembranosus*) using a meat stretching device. *Meat Science*, (Submitted).

Toohey, E. S., van de Ven, R., Thompson, J. M., Geesink, G. H., & Hopkins, D. L. (2011). SmartStretch™ Technology. II. Improving the tenderness of leg meat from sheep using a meat stretching device. *Meat Science*, (Submitted).

Toohey, E. S., van de Ven, R., Thompson, J. M., Geesink, G. H., & Hopkins, D. L. (2011). SmartStretch™ Technology. III. The impact of medium voltage stimulation

and SmartStretch™ technology on sheep topside (*M. semimembranosus*) meat quality traits under commercial processing conditions. *Meat Science*, (Submitted).