

Effect of automated *pre rigor* stretching on beef tenderness development

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Table of Contents

Table of Contents	i
List of Figures	iii
List of Tables	iv
Attestation of Authorship	v
Acknowledgements	vi
Abstract	vii
Chapter 1 Introduction	1
1.1. Importance of tenderness	1
1.1.1. Consumer acceptability	1
1.2. New Zealand beef industry	1
1.2.1. New Zealand beef production and slaughter.....	1
1.2.2. New Zealand animal production for meat.....	2
1.3. Structure of skeletal muscle	3
1.4. Conversion of muscle to meat	7
1.5. Factors contributing to tenderness	9
1.5.1. Connective tissue.....	9
1.5.2. Muscle shortening and sarcomere length	11
1.5.3. Cold shortening and heat shortening.....	13
1.5.4. Ageing and proteolysis.....	14
1.5.5. Intramuscular fat	19
1.6. Strategies to improve meat tenderness.....	20
1.6.1. Cooking	20
1.6.2. Electrical stimulation.....	21
1.6.3. Mechanical tenderisation.....	23
1.6.4. Exogenous enzymes	26
1.6.5. Tenderstretch.....	26
1.6.6. Tendercut.....	29
1.6.7. Wrapping.....	30
1.6.8. SmartStretch™/Smartshape™	32
1.7. Objective of this thesis.....	35

Chapter 2 Materials and Methods	36
2.1. Materials and equipment.....	36
2.1.1. Muscles.....	36
2.1.2. Chemicals and solutions.....	36
2.1.3. Equipment	36
2.2. Methods	39
2.2.1. Animals and treatments	39
2.2.2. Measurements.....	39
2.2.2.1. Purge loss.....	39
2.2.2.2. pH	40
2.2.2.3. Drip loss.....	40
2.2.2.4. Cooking loss and shear force.....	40
2.2.2.5. Sarcomere length	41
2.2.2.6. Western blot to determine desmin degradation	41
2.2.2.7. Data analysis.....	45
Chapter 3 Results and Discussion	46
3.1. pH, loin length and sarcomere length	46
3.2. Shear force	48
3.3. Water-holding capacity.....	52
3.4. Proteolysis (desmin degradation).....	53
Chapter 4 Conclusion.....	55
References.....	56
Appendix 1.	65

List of Figures

Figure 1.	Schematic representation of the structural organisation of muscle from subcellular myofibrils to whole organ.	4
Figure 2.	A small part of the sarcoplasmic reticulum is shown inside part of a muscle fibre.	5
Figure 3.	Structural arrangement of the sarcomere	6
Figure 4.	Illustration of the sliding filament theory: relaxation and contraction. Thin filaments and thick filaments interdigitate allowing for sliding of filaments past each other.	7
Figure 5.	(a) Rows of needles or blades in a large mechanical tenderizer, (b) A hand-held tenderiser (Jaccard™).	24
Figure 6.	Impact of mechanical tenderisation on the tenderness of cuts from beef carcass	24
Figure 7.	Schematic drawing of Tenderstretch.	27
Figure 8.	Schematic drawing of the Tendercut system with a cut in the 12th/13th vertebrae region of a beef carcass side	29
Figure 9.	Schematic drawing of the Pi-Vac packaging system for hot-boned muscles; (A) start position and (B) after filling the machine	32
Figure 10.	SmartStretch™/SmartShape™ machine	33
Figure 11.	SmartStretch™/SmartShape™ concept.	34
Figure 12.	The water bath used to cook meat samples.	37
Figure 13.	Microscope for sarcomere length measurement	38
Figure 14.	(a) G2 tenderometer (b) MIRINZ tenderometer	38
Figure 15.	Layout of 96-well to determine protein concentration	42
Figure 16.	Transfer of protein to membrane by Western blotting, showing the arrangement of the blotting ‘sandwich’	44
Figure 17.	A representative photo of control and stretched loins	47
Figure 18.	Relationship between sarcomere length (µm) and the percentage increase in length	48
Figure 19.	Mean shear force (kgF) of stretched (4S) and control (CON) samples for 1, 7 and 14 days <i>post mortem</i> measured with MIRINZ and G2 tenderometers	49
Figure 20.	Relationship between shear force (kgF) and sarcomere length (µm)	51
Figure 21.	Representative Western blot desmin of whole muscle protein extracts from <i>M. longissimus</i> with control (CON) and stretched (4S) loin samples at different <i>post mortem</i> ageing periods (Days 1 and 14)	53

List of Tables

Table 1.	Means of pH, purge loss, drip loss, cooking loss and sarcomere length at different <i>post mortem</i> days (1, 7 and 14 days) for SmartStretch TM /SmartStretch TM (4S) and control (CON) loins.....	47
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Attestation of Authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

Signed:

A handwritten signature in blue ink, consisting of stylized, overlapping loops and a long horizontal stroke, positioned above a thin horizontal line.

Date:

February 2102

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Abstract

This thesis discusses the effects of *pre rigor* stretching technology by SmartStretchTM/SmartShapeTM (4S) technology on beef tenderness development in *M. longissimus dorsi*.

Twelve steers (approximately two years of age) were selected and slaughtered at the Ruakura abattoir under a standard slaughtering process. Both loins (*M. longissimus dorsi*) from all animals were randomly allocated into two treatments [stretching (4S) or control] (left or right) depending upon sides and three ageing times (1, 7 and 14 days of ageing). Assigned stretching loins were stretched with a 4S machine (Fix-All Services Company Ltd, Hamilton, New Zealand) whereas non-stretched loins (controls) were wrapped in cling polyethylene film. Initial and final lengths of 4S stretched loins were measured. The entire sampling and treatment process was conducted in approximately around one hour and then all meat loins were transported to a laboratory for storage at -1.5°C.

At 24 hours *post mortem*, the length of the stretched loins was measured again and all meat loins were cut into three sections in preparation for three different ageing times. The meat sections of 7 and 14 day aged loins were weighed to determine for purge loss measurement prior to vacuum packaging. At the assigned ageing times (1, 7 and 14 days of ageing), each loin was separated into five parts for different measurements including shear force (two tenderometers), sarcomere length, drip loss and biochemistry analysis (proteolysis). The meat loins aged for 7 and 14 days were reweighed to determine purge loss.

The results showed that there was on average a 33% increase in length by 4S and there was no significant difference in pH value due to the 4S treatment. In addition, there was no significant difference for sarcomere length between treatments. Shear force values were not influenced by 4S ($P > 0.05$); however, ageing contributed to an improvement in tenderness ($P < 0.05$). Moreover, there was no significant effect of the 4S treatment on water-holding capacity of loins.

A Western blot assay showed that there was no stretching effect on extent of proteolysis of desmin degradation early *post mortem*; however, with ageing apparent evidence on muscle protein degradation was observed. For a fuller picture of the value of 4S treatment, the 4S technology should be applied to a range of beef muscles to attain with different percentage

increases in length, types of cattle, either electrical stimulated or not, various pH values, different ageing times and temperatures to explore optimal extent of stretching to achieve maximum meat tenderness.

Chapter 1

Introduction

1.1. Importance of tenderness

1.1.1. Consumer acceptability

There are three main meat attributes that govern consumers' eating satisfaction. These are tenderness, juiciness and flavour (Miller, 2003). Tenderness is the ease to bite or chew a piece of meat in the mouth. Juiciness refers to the quantity of apparent juice that comes from meat during chewing while flavour represents the interaction between aromatic compounds within the nasal cavity and chemical compounds that contact the tongue during eating. These three attributes have been defined and explored by a number of subjective and objective methods, such as degree of liking and ranking preference (Miller, 2003).

Of the three, tenderness has long been considered as one of the most important eating factors which strongly affects the consumer's willingness to spend money on perceived meat quality. The meat industry has been developing means by which skeletal muscle is processed, produced and modified to yield meat that is optimally and consistently tender. This is not easy to achieve because muscles are heterogeneous within an animal and there is the usual biological variability between animals. For this reason, 'tenderness is the major problem facing the meat industry in the future of the huge US beef industry (Lusk, Fox, Schroeder, Mintert & Koohmaraie, 2001).

1.2. New Zealand beef industry

1.2.1. New Zealand beef production and slaughter

In the agricultural sector, the red meat industry is one of New Zealand's major export earners, generating revenues to the New Zealand economy of more than NZ\$ 7 billion every year (Meat Industry Association, 2011). Red meat and its co-products comprise 23% of total export value of New Zealand's primary sector, and have recently been increasing. This is due to the higher requirement of global demand for protein sources and producer-level fluctuations by the main exporting nations, in particular for sheepmeat (Meat Industry Association, 2011). By the end of June 2011, New Zealand beef exports experience a slight decrease compared to previous year. Nevertheless, beef export figures

reveal more income (NZ\$ 200 million) than the previous year, growing to more than NZ\$ 2 billion income (Meat Industry Association, 2011).

More than 80% of New Zealand beef production is exported (Meat Industry Association, 2011). The largest market for New Zealand exported beef is North America, with just less than half of total New Zealand beef export by volume and by value. The next largest market is North Asia (Japan, South Korea, and Taiwan) taking 23 % by volume of overall beef exported (92,000 tonnes) to these countries. The third market is South East Asia which accounts for more than 10% by volume (43,000 tonnes). Another significant market is the European Union with 4 % by volume (Meat Industry Association, 2011).

The cattle slaughter in New Zealand is grouped into five major categories. These are bull, boner cow, heifer and castrated bull (together called 'prime'), and veal. In terms of head of cattle, this slaughtering number respectively contributes to an increase in slaughter numbers by 2.28 million head (Beef + Lamb New Zealand, 2011). Cow slaughter represents the dominant number with a 5.7% increase compared to previous year and followed by steer. Bull and heifers account for a third and a fourth main contributions to the beef slaughter respectively. By the end of June 2011, total beef cattle number is approximately 3.94 million head in which this value remains constant compared to previous year. Most beef cattle in New Zealand are raised in the North Island, where the total number is around 2.81 million head. However, this number has declined due to the development of the dairy industry. Indeed, beef cattle numbers increase in the South Island representing of 1.13 million head. Dairy cattle comprise 6.10 million heads by the end of June 2011 (Beef + Lamb New Zealand, 2011).

1.2.2. New Zealand animal production for meat

Almost all New Zealand sheep, cattle and deer raised on New Zealand pasture are primarily finished on a diet largely consisting of perennial ryegrass and clover, the latter a legume that is useful for nitrogen fixation in New Zealand's mineral deficient soils (Meat Industry Association, 2009). The fat of pasture-fed animals contains relatively high levels of omega-3 fatty acids that are often found deficient in grain-fed diets. The meat from grain-fed cattle is also generally low in fat (Wood et al., 2008). Many food manufacturers around the world order red meat from New Zealand because of its high hygiene standards, and its guaranteed freedom from bovine spongiform encephalitis and similar prion diseases

(Meat Industry Association, 2009). Additionally, because New Zealand meat is often exported chilled, meat ageing over the weeks in transit usually yields a tender product (Meat Industry Association, 2009). However, in the domestic market and sometimes in the export markets, New Zealand meat is not tender, so tenderness remains a research focus. From any slaughter animal, meat is muscle in *rigor* at early *post mortem*. To address the issue of meat tenderness in this thesis, it is first important to understand the structure of muscle during the conversion of muscle to meat.

1.3. Structure of skeletal muscle

Each skeletal muscle is different from another by size and shape, but at a cellular level all skeletal muscles are composed of parallel organization of lengthened, multinucleated cells, called muscle cells or muscle fibres or myofibres (Strasburg, Xiong & Chiang, 2007). Depending on food animal species, animal muscle and variation within the muscle, the width of each myofibre is between 10 and 100 μm and from 1 and 2 mm to many centimetres long and sometimes spanning the whole muscle length. Myofibres are organized in a hierarchical structure (Figure 1). The individual myofibres are covered by a coating of connective tissue called endomysium and collections of myofibres are arranged in primary and secondary bundles that are separated by another layer of connective tissue known as perimysium (Strasburg et al., 2007). The last coating of connective tissue sheaths the entire muscle called epimysium. These sheaths combine with connective tissue muscles to connect muscles with bones (Strasburg et al., 2007). Connective tissues (perimysium and endomysium) join together to offer a beneficial structure to sustain structural integrity for muscle during resting or contracting states. Adipose tissue which is frequently associated with connective tissues may also keep to maintain structural integrity of muscle (Aberle, Forrest, Gerrard & Mills, 2001).

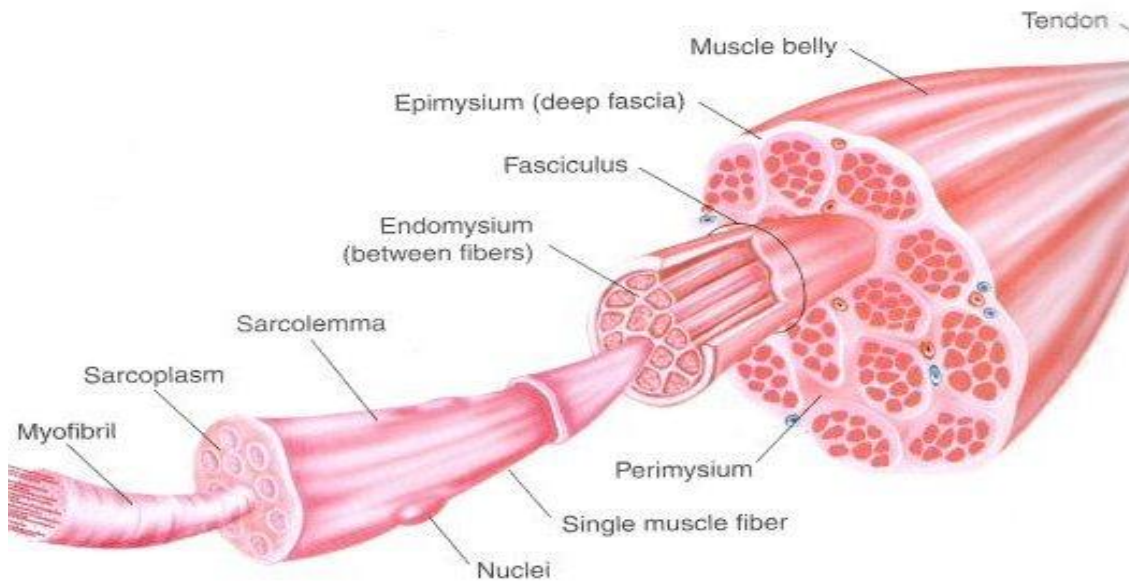


Figure 1. Schematic representation of the structural organisation of muscle from subcellular myofibrils to whole organ. Individual muscle cells (fibres) are surrounded by a layer of connective tissue (endomysium) which in turn are organized into bundles (fascicles), separated by a layer of connective tissues called the perimysium (Baechle & Earle, 2008).

Muscle is permeated with a complicated structure of nerves that govern the control of muscle contraction by way of invaginations of the muscle cell's membrane, the sarcolemma. The invaginations are the transverse tubules or T-tubules that transversely transfer electric signals for contraction to internal parts of the myofibre. Through a so-called triad structure the signal is transferred to longitudinally oriented intracellular membrane network known as sarcoplasmic reticulum (Figure 2). The sarcoplasmic reticulum system surrounds the myofibrils and serves as calcium reservoirs to control muscle contraction. Other proteins in the sarcoplasmic reticulum act to regulate calcium ion concentration by pumping calcium in or out depending on the contractive state of the muscle cell (Dulhunty, Haarmann, Green, Laver & Casaratto, 2002; Rossi & Dirksen, 2006).

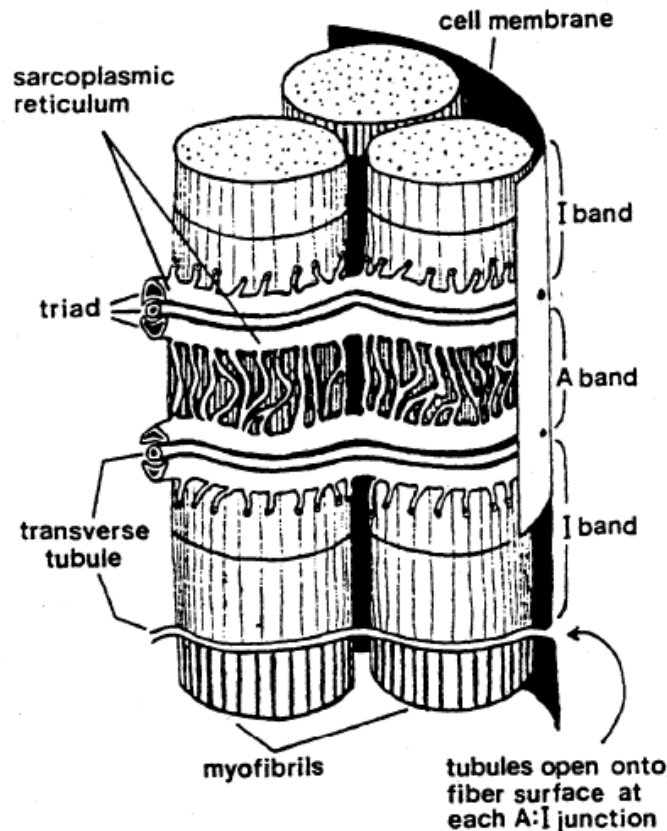


Figure 2. A small part of the sarcoplasmic reticulum is shown inside part of a muscle fibre (Anonymous, n.d.).

Muscle cells are multinucleate cells. The nuclei are spread out and are mostly found under the sarcolemma. Another organelle is the mitochondrion, the primary source of oxidation-derived energy for contraction and other cellular activities. A high number of mitochondria are present in oxidatively active muscle cells. Oxidative metabolism is supported by cytoplasmically-located protein called myoglobin. This protein is chemically similar to the haemoglobin of red blood cells, and acts as a mean to store and to transfer oxygen to mitochondria (Strasburg et al., 2007). Yet another organelle is the lysosome which plays a crucial role as the main storage organelle for proteolytic enzymes including cathepsins for catabolic functions (Strasburg et al., 2007).

Muscle contraction is caused by action of cytoskeletal proteins that are assigned into parallel, thin and thick filaments (myofilaments). These filaments occupy from 80% to 90% of myofiber by volume. The myofilaments are organized into myofibrils that play a crucial role in coordinated integrity as contractile elements of a muscle cell (Figure 3). Early studies on muscle structure with conventional transmission light microscopy revealed a distinct repeating banded (striated) structure. Polarised light microscopy shows

more detail of repeating light and dark bands that are the fundamental structural unit of muscle contraction. These are the sarcomeres (Figure 3). The dark bands are anisotropic under polarised - A- bands - and the light bands - I-band - are isotropic. The sarcomere length is between one Z-disc and an adjacent Z-disc in a myofibril. Z-disc is narrow, and a dark band of proteins (electron-dense in transmission electron microscopy) in the centre of the I-bands. The proteins in Z-disc act as anchoring structure; thin filament proteins stem from both sides of Z-disc (Strasburg et al., 2007).

The thin filaments overlap thick filaments within the A-band. The centre of the A-band centre is slightly less dense than distal regions and thus looks brighter since this point contains only thick filaments, known as H-zone. At the H-zone centre is another line called M-line, are dark zone that runs parallel to the Z-disc. M-line comprises of varying proteins that retain structural organization of thick filaments and functions as a anchoring position for proteins in which cross from M-line to Z-disc (Strasburg et al., 2007).

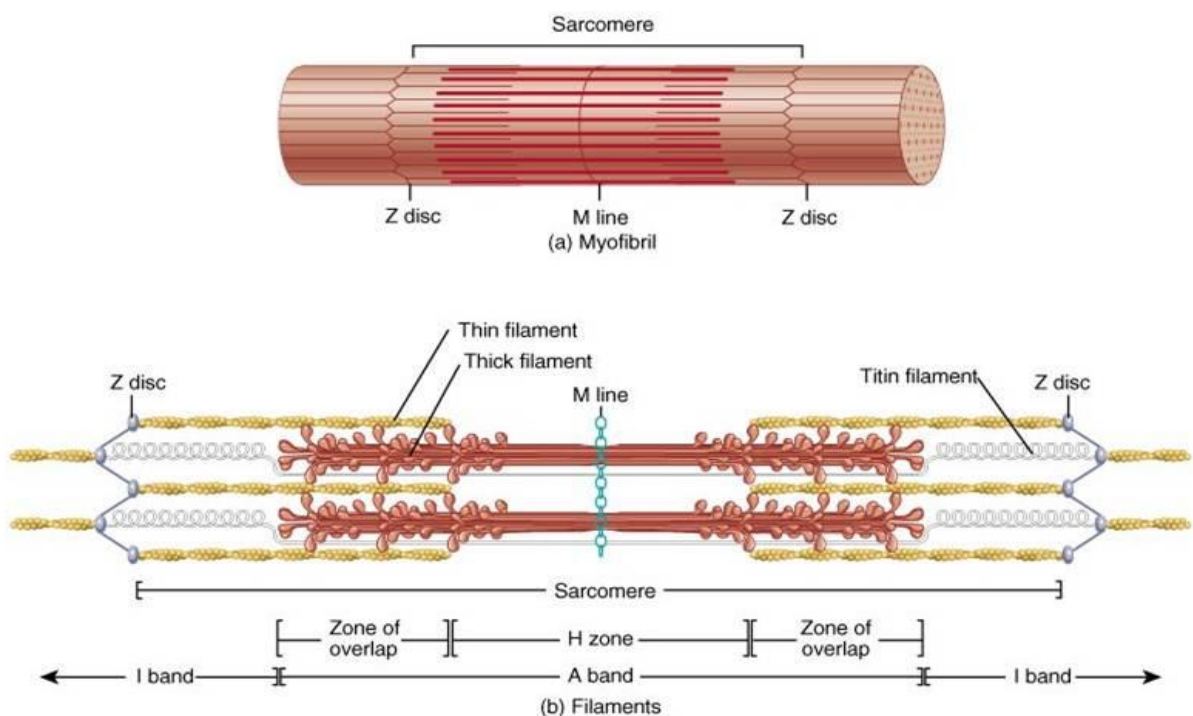


Figure 3. Structural arrangement of the sarcomere. (a) The sarcomere unit of a myofibril begins at one Z-disc and extends to next Z-disc. (b) The major components of the sarcomere are thin filaments, which are anchored at the Z-disc, thick filament in the central region of the sarcomere that partially overlaps with the thin filaments, and titin filaments that span from the Z-disc to the M-line (Tortora & Derrickson, 2006).

In exploring the mechanism of muscle contraction, Huxley and Hanson (1954) proposed the sliding filament theory. This theory was based on the fact that the lengths of thick and thin filaments stayed stable, not dependent on either muscle was stretching, contracting or in resting status. However, the sarcomere lengths altered with stretch and contraction (Figure 4). Cross-sectional (transverse plane) electron micrographs showed that thin filaments were hexagonally arranged (but parallel to) around thick filaments. When contraction was occurred, thick and thin filaments slid each other in that way thin filaments moved toward each other. Thus, the muscle sarcomere length was shortened. In contrast, stretching effect caused an increase in sarcomere length because thin filaments moved away from the A-band. As will be discussed (1.5.2) and determined later, the degree of overlap of thick and thin filaments can have a marked effect on meat tenderness.

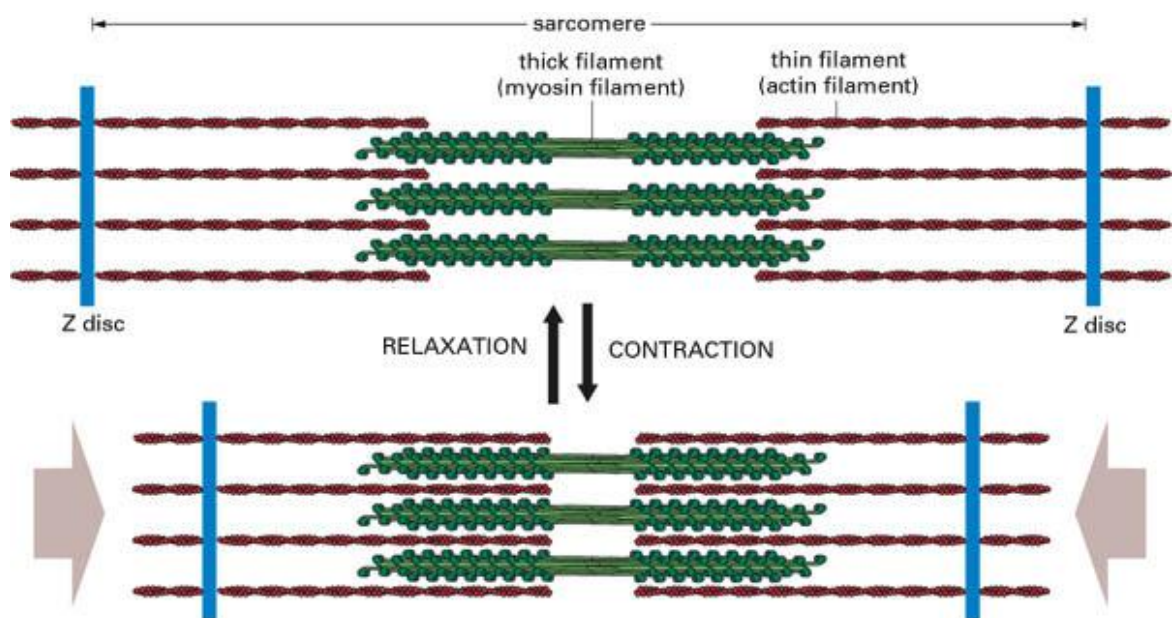


Figure 4. Illustration of the sliding filament theory: relaxation and contraction. Thin filaments and thick filaments interdigitate allowing for sliding of filaments past each other (Tortora et al., 2006).

1.4. Conversion of muscle to meat

Living muscle tissue has to sustain functions by generating high energy compounds. Adenosine triphosphate (ATP) represents the most important source of energy for all metabolic activities, homeostasis and muscle contraction. The circulatory system provides oxygen and nutrients to generate energy and also to transport metabolic products from or into muscle cells (Strasburg et al., 2007). To convert muscle to meat, main biochemical processes are done in favour of muscle attaining *rigor mortis* (Honikel, 2004). A key

process *post mortem* is the hydrolysis of ATP in muscle to energize and maintain the structural integrity of muscle. Ongoing glycolysis eventually leads to the depletion of glycogen (Immonen & Poulanne, 2000) which coincides with the depletion of ATP. When *post mortem* glycolysis reaches ultimate termination coinciding with a depletion of ATP, thus the muscle commences to shorten to some extent that depends on temperature and pH of muscle (Locker & Hagyard, 1963). While amount of ATP is low in muscle, ability of the calcium pump protein is diminished to retain sarcoplasmic calcium concentration in the resting muscle concentration range. Similarly, the number of myosin cross-bridge is gradually increased with actin that stays locked due to a shortage of ATP to dissociate these both proteins (Rawn, 1989) and this phenomenon leads to muscle turning to be inextensible.

When an animal is slaughtered, blood is no longer transferred to the muscle to support metabolic functions. All organs including muscles utilize their reserved components to activate other important functions to sustain cellular homeostasis. In the case of muscle, when there is no more oxygen available from the circulatory system, the myofibres use remaining oxygen bound to myoglobin to maintain aerobic metabolism generating ATP. That oxygen is rapidly depleted, and ATP is subsequently derived from creatine phosphate and ADP by the enzyme creatine kinase (Strasburg et al., 2007). When that pool of creatine phosphate is exhausted, ATP is then generated from glycogen by anaerobic glycolysis and also generates in lactate. However, the pool of glycogen is also finite and the ATP concentration soon falls below the concentration required homeostasis, about 5 mM. As the ATP concentration declines and it is accompanied by an increase in the concentration of H^+ leading to a fall in pH that accompanies lactate accumulation. Another role of ATP is used to power the Ca^{2+} ion pumps that remove calcium from the cytoplasm into the sarcomplasmic reticulum. Thus muscles tend to contract (see also 1.5.2) and consequently consume more ATP. Muscle remains stretchable as long as ATP concentrations are above 1 mM. However, below 1 mM the myosin heads lock with the actin in thin filaments and, rendering the muscle inextensible. This is the *rigor* state, known as *rigor mortis* (Strasburg et al., 2007). The time taken to achieve *rigor mortis* depends on muscle, species, genetics, nutritional status, management before slaughter and slaughter process (Lawrie, 1998). For mammalian slaughter animals to reach the *rigor mortis* takes minutes (highly stressed pigs) or up to 24 hours (unstressed, well-nourished

bovines carcass-cooled quickly). In poultry the time typically ranges from 30 minutes to 2 hours (Lawrie, 1998).

It is noted above that muscle pH declines after slaughter. The extent of fall is limited for a number of reasons, but principally due to the limited concentration of glycogen in muscle. In the case of bovines, when muscle glycogen concentrations are maximally high - about 1.5% in the live animal - the pH will fall from 7 to about 5.5 in *rigor*. However, where muscle glycogen concentration is severely limited pre slaughter, *post mortem* muscle pH can be as high as 7 (Honikel, 2004).

The last stage of conversion of muscle to meat is ageing or the 'resolution of *rigor*'. This greatly improves the eating quality in terms of cooked meat tenderness. Meat tenderness attained during ageing is due to endogenous proteolytic enzyme activity, and the extent and rate at which meat tenderises depending on muscle, species, genetics, nutritional status, management before slaughter and slaughter process. In cold-stored meat, beef can take weeks to achieve acceptable tenderness (Geesink, 1993; Koohmaraie, Shackelford & Wheeler, 1998).

1.5. Factors contributing to tenderness

1.5.1. Connective tissue

Muscle connective tissue is a trace component consisting around 1 to 4 % of dry weight in many muscles with roles as fibre adhesion, force transmission, tissue arrangement and outer protection (Taylor, 2004). The connective tissues offer physical support and through the associated blood supply (arterioles, capillaries and venules), the means to transport nutrients and waste products to and from the muscle fibres. Connective tissues contain inflexible cells, free cells and proteins that they are organized in one matrix (Davies, 2004). The inflexible cells comprise fibroblasts which generate fibrous proteins and a connective tissue matrix in arrangement analogous to steel-reinforced concrete. The 'concrete' of collagen is a complex mixture of glycoproteins and proteoglycans, that varies with collagen expression (Pedersen, Kolset, Sorensen & Eggen, 1999).

Connective tissues are arranged in a hierarchal manner: the endomysium encases muscle fibres and comprises a basal lamina and minor amount of collagen. The endomysium is similarly arranged in all muscle types. The endomysium's role is to fibre-to-fibre

connection and force transmission. The next level in the hierarchy is the perimysium, which surrounds groups or bundles of fibres. Finally, the epimysium surrounds the entire muscle being as a sheath of connective tissue (McCormick, 1999; Taylor, 2004).

Two major proteins of connective tissues are collagen and elastin. The content of elastin is less than 0.4% of dry weight except in the *semitendinosus* and *latissimus dorsi* muscles where it accounts for 2% of dry weight (Bendall, 1967). Collagen comprises a repeating polypeptide sequence typically pattern Gly-Pro-X or Gly-X-Hyp, where X may be any of various other amino acid residues. Unlike other proteins collagen thus contains high concentration of hydroxyproline, approximately 13% in warm-blood mammals (Lawrie & Ledward, 2006). The particular amino acid sequence gives rise to helical structure in individual collagen molecules that are arranged in a tertiary triple helix. Collagen muscle can be elastically stretched to increase in length by 4%, without permanent deformation. In this range it is highly elastic, ranging from 500 to 1,000 MPa, and it is thus highly flexible. Elastin by contrast is not so rigid with an elasticity of 0.1 to 0.4 MPa (Siver, Kato, Ohno & Wasserman, 1992). Collagenous tendons are more likely to stretch around 4% of their length elastically and they return to their own initial length resulting in no permanent deformation. Indeed, elastic fibres comprises elastin in which its polypeptide chains are sequenced randomly (Davies, 2004).

There are many types of collagen. Collagen types I, III and IV dominate muscle connective tissue (Listrat, Lethias, Hocquette, Renand, Mennissier & Geay, 2000), and each has a distinct amino acid sequence. Endomysium comprises collagen IV, proteoglycans, basal lamina proteins and lesser quantity of collagens I and II while perimysium comprises collagen I and III in proportions that vary with animal age. Collagen type I is dominant in epimysium (Lawrie et al., 2006). Locomotive muscles are more likely to contain higher collagen concentrations than positional muscles (Taylor, 2004). Torrescano, Sanchez-Escalante, Gimenez and Roncales and Beltran (2003) measured the collagen concentration in 14 bull bovine muscles and found that the lowest concentration was in the *psoas* (a positional muscle) and highest in the *flexor digitorum* muscles (a locomotive muscle).

Collagen is generally considered as ‘the background toughness’ of mammalian muscles, meaning that the tenderisation that proceeds with time of meat storage has no effect on

toughness due to collagen (Etherington, 1987). It is responsible for ‘enduring toughness’ in situations where the collagen does not denature and thus doing so collagen melts into amorphous gelatine. As an animal ages its collagen matures by the development of inter and intramolecular cross-links that stabilise collagen against heat denaturation (Young & Gregory, 2001). Researchers believe that hydroxyaldohistidine and pyridinoline cross-links serve as major roles for this mechanism (Lawrie et al., 2006). For young animals, immature collagen is converted to gelatine at 60°C and gelatine is not responsible for toughness. However, meat from older animals requires extended cooking and/or higher temperature to denature the collagen. Thus meat with a high collagen concentration and from mature animals is unsuited to rapid cooking, but well suited to casserole dishes.

1.5.2. Muscle shortening and sarcomere length

Locker and Hagyard (1963) showed that if unrestrained beef *sternomandibularis* muscle entered *rigor* outside a temperature range of 14 to 19°C, the muscle should shorten, more so at lower temperatures than higher temperatures. Muscle was shortened due to muscle contraction as the contractile proteins myosin (thick filament) and actin (thin filaments) slid over each other leading to shorter sarcomere length (Herring, Cassens, Suess, Brungardt & Briskey, 1967). When cooked, the shortened muscles were tougher than unshortened muscles. This discovery suggested that sarcomere length had an important effect on tenderness.

Many experiments have been subsequently conducted to examine the relationship between sarcomere length and shear force and indicator of tenderness. Herring, Cassens and Briskey (1965) reported that longer sarcomere lengths of muscle contributed to lower shear force values compared to shorter sarcomere lengths. Marsh and Leet (1966) examined the relationship between shortening and tenderness. They found that 20% shortening of the initial excised length did not cause a significant toughening effect, but toughness increased rapidly with further shortening beyond this value, reaching a peak of several times its original value at a shortening of about 40%. On further shortening, the meat became progressively more tender until at about 55 to 60% shortening it was cleaved about as easily as unshortened meat. A model for this reversal of toughness with extreme shortening was described by these authors: at 40% contraction the thick and thin filaments were maximally overlapped, but beyond this degree of contraction the thick filament penetrated in the Z-disc resulting in massive destruction of muscle structure and thus

leading to tenderisation. The work of Marsh and Leet (1966) was supported by Bouton, Fisher, Harris and Baxter (1973a) and Hostetler, Link, Landmann and Fitzhugh (1972). However, other workers found no relationship or contradict relationships between sarcomere length and shear force (Parrish, Vandell & Culler, 1979). Work by Marsh and Carse (1974) investigated the association between muscle shortening and tenderness. Results demonstrated toughness of muscle reached peak with 35% shortening whereas 25 to 30% stretched muscle resulted in less tough muscle. Thus, these results indicated that initial sarcomere length of this work was determined in accordance with percentage of muscle shortening resulting from initial carcass length rather than real measurement of sarcomere length.

More recently, Smulders, Marsh, Swartz, Russell and Hoenecke (1990) investigated the impact of sarcomere length on meat tenderness for bovine *M. longissimus dorsi* from aged and unaged carcasses. The result showed that if the pH was less than 6.3 at 3 hours *post mortem*, there was no association between sarcomere length and tenderness for both categorical carcasses. If the pH was higher than 6.3 at the time, there was a strong association between sarcomere length and tenderness. They proposed that tenderness was strongly related to shortening for a slow-glycolysing carcass; however, this association was not present in muscle from a rapid pH fall or fast-glycolysing carcass.

One study from Wheeler and Koohmaraie (1994) on sheep *longissimus* muscle reported that reduced sarcomere length contributed to increased toughness occurring at onset of *rigor* during 0 to 24 hours *post mortem*. Specifically, meat reached peak toughness during 9 to 24 hours after slaughter and tenderness variability was apparently evident after 1 day of ageing (Koohmaraie, Doumit & Wheeler, 1996; Savell, Mueller & Baird, 2005). However, Koohmaraie (1996) stated if sarcomere shortening was restricted at the onset of *rigor*, toughening was reduced.

Therefore, the effects of shortening are variable, but overall the industry view is that it should be avoided. This is evident from the fact that accelerated glycolysis, temperature control techniques and certain mechanical techniques are commercially applied to either minimise shortening or to actively stretch muscle. These techniques would not be applied if the benefit, real or perceived, did not exceed the cost associated with these techniques. The techniques include electrical stimulation to accelerate glycolysis, Achilles hanging,

Tenderstretch, Tendercut, wrapping and SmartStretchTM/SmartShapeTM (4S) technology. These techniques will be discussed in more detail in later sections.

1.5.3. Cold shortening and heat shortening

In the early 1960s the New Zealand lamb industry had a meat toughness issue raised from retailers in Europe and North America. The problem was traced to a too rapid cooling process after slaughter that led to severe muscle shortening in lamb carcasses destined to freezing and export. The phenomenon became known as ‘cold shortening’.

A plausible mechanism of cold-induced shortening has been described by Pearson, Carse, Davey, Locker and Hagyard (1973) and other associated workers. Cold shortening results from *pre rigor* contraction due to a high concentration of calcium (as Ca^{2+}) in muscle cells, that is responsible for activating the myosin ATPase. The cold temperatures reduce the efficiency of the calcium pumps needed to pump calcium out of the muscle cell into the sarcoplasmic reticulum (Bendall, 1978) where it is inactive from a contraction perspective. Generally, the conditions that induce cold shortening are when muscle temperature is below 10°C, pH is over 6.2, and the muscle is in the *pre rigor* state (Dransfield, 1994). (In the high pH-dark cutting-condition, a muscle can be in *rigor* above pH 6.2 if the glycogen concentration at slaughter is low enough.) Muscle containing a higher proportion of red muscle fibres (Type I) (Young, 1984) are more prone to cold shortening. These have a less efficient calcium pump than Type II fibres for reabsorption of calcium from the muscle cells into the sarcoplasmic reticulum (Pearson & Young, 1989). These are slower to enter *rigor* such that at 3 hours *post mortem* the pH is more likely to be greater than pH 6.3 (Smulders et al., 1990). White muscles, rich in Type II fibres, decline in pH faster and are less prone to cold shortening. Thus poultry muscles from chickens are less likely to cold shorten than redder muscle from ruminants (Strasburg et al., 2007).

The obvious solution to the cold shortening is to minimise cooling in the early stages of *rigor* attainment, but that conflicts with the hygiene demand of rapid cooling. This conflict can be resolved by electrical stimulation, and this is discussed later.

At a temperature well above 20°C, muscle may also undergo ‘heat shortening’ or ‘*rigor* shortening’ (Lee & Ashmore, 1985). *Rigor* shortening contributes to muscle being tough that is resulted from isometric tension and diminished proteolytic enzyme activities

(Devine, Payne & Wells, 2002b; Hwang & Thompson, 2001). High temperatures are likely to accelerate the glycolytic rate because of enhanced action of glycolytic enzymes contributing to increased rate of pH decline. Interaction between faster pH decline and higher temperature contribute to the shortening conditions. Muscles are exposed to high temperature that induces in high calcium ion concentration being released because high concentration of calcium plays a key role at onset of *rigor mortis*; therefore, muscle commences contracting due to *rigor* shortening. Mitochondria that release calcium ions would not function if ATP content still exists (Mickelson, 1983). As a result, contraction-induced *rigor* shortening happens at onset of *rigor mortis* rather than before onset of *rigor mortis* (Hwang, Devine & Hopkins, 2003). Compared to cold shortening, *rigor* shortening is less rigorous due to less ATP content to generate contraction.

Muscle temperature had impact on the relationship between stretching and meat tenderness (Bruce & Ball, 1990; Devine et al., 2002b; Devine, Wahlgren & Tornberg, 1999). While muscle remained at temperature of 15°C for the duration of *rigor mortis*, it contributed to reduction of sarcomere shortening coinciding with improved tenderness. However, muscle was at onset of *rigor mortis* temperature (over 30°C) generated tough meat. Therefore, optimum temperature maintaining muscle at onset of *rigor mortis* was at 18°C since this temperature provided facilitating condition for meat to be equally tender, irrespective of sarcomere shortening (Devine et al., 2002b).

In addition, another study investigated effect of different temperatures during *rigor* on meat quality; results revealed that *rigor* temperature at approximately 15°C provided the longest sarcomere length and the lowest shear force values (Geesink, Bekhit & Bickerstaffe, 2000).

1.5.4. Ageing and proteolysis

Ageing refers to tenderness development in meat that occurs while meat is held in storage. The factors influencing tenderness development has been studied for several decades since the pioneering work (Davey & Gilbert, 1976). *Post mortem* tenderization is due to the activities of proteolytic enzymes that hydrolyse selected myofibrillar and cytoskeletal proteins to loosen structural integrity of muscle (Ouali, 1992; Sentandreu, Coulis & Ouali, 2002).

There are three major protease classes thought to be involved in proteolysis. These are ubiquitin proteasome, lysosomal enzymes and the calcium-activated proteases, with the first two groups believed to be most responsible (Huff-Lonergan, Mitsuhashi, Beekman, Parrish, Olson & Robson, 1996; Koohmaraie, 1988). The lysosomal enzymes-the cathepsins-are believed to be most active under acid conditions, such as when an ultimate pH below 6 is attained. However, most studies generally agree that calpains play one of the most important roles in ageing (Huff-Lonergan et al., 1996; Koohmaraie, 1988). The calpain group consists of μ -calpain and m-calpain and their inhibitor (calpastatin). μ -Calpain and m-calpain are ubiquitous tissue enzymes that respectively require micromolar and millimolar calcium ion concentration to activate their actions (Goll, Thompson, Li, Wei & Cong, 2003). μ -Calpain is the most important enzyme for cytoskeletal proteins *post mortem* because of its lower requirement of calcium ion concentration for activation. In *post mortem* muscle the Ca^{2+} concentration in the sarcoplasm rises from 10^{-7} M to around 10^{-4} M because of the failure of the calcium pump in the sarcoplasmic reticulum (Vidalenc, Cottin, Merdaci & Ducastaing, 1983). The activity of calpains is not only governed by calcium concentration but also by the concentration of calpastatin, the endogenous inhibitors of the calpains (Wheeler, Savell, Cross, Lunt & Smith, 1990).

Whatever enzymes and enzyme inhibitors are involved in protein degradation, they have to hydrolyse the muscle proteins which are responsible for muscle cell integrity. These cytoskeletal proteins can be intermyofibrillar (desmin), intramyofibrillar (titin and nebulin), and also proteins that link proteins to sarcolemma and cell membrane (dystrophin and vinculin) (Koohmaraie, 1996). The extent of desmin degradation is considered as one key element of factors in the *post mortem* tenderisation in muscles, because desmin serves a crucial role to retain structural integrity of muscle cells. Desmin connects the myofibrils to the Z-disc altogether and the peripheral layer of myofibrils to the cellular structure, this could contribute to meat tenderness (Robson, 1991). For example, Huff-Lonergan, Mitsuhashi, Beekman, Parrish, Olson and Robson (1996) found that desmin was degraded more slowly in myofibrils of meat samples with higher shear force values than lower values.

The rate and extent of ageing are affected by several factors including animal species, breed within species, muscle types, animal growth rate, pre slaughter handling, electrical

stimulation, chilling rate and its associated temperature regime, and ultimate pH (Farouk, Wiklund & Rosenvold, 2009).

Smulders, Barnier, Geesink and van Laack (1995) found that the ageing rate of meat from different species of slaughter animals was in the order: chicken > pork > venison > lamb > beef, and that this order reflected the *post mortem* glycolysis rates. The ageing time to develop tenderness classically occurred in 1 to 2 days for chicken, 3 to 6 days for pork and 10 to 20 days for beef (Smulders, Toldra, Flores & Prieto, 1992). Differences between species are well illustrated by a study comparing between venison and beef. Farouk, Beggan, Hurst, Stuart, Dobbie and Bekhit (2007) studied on *semimenbranosus* from red deer (*Cervus elaphus*) venison and beef and they found that venison was acceptably tender whereas beef was very tough at 24 hours *post mortem* stored at -1.5°C. After 7 days of ageing, venison samples were very tender but beef samples achieved this point requiring 21 days of ageing. Previous studies on ageing in reindeer (*Rangifer tarandus tarandus*) and beef showed that *longissimus* muscle from deer was very tender as quickly as 3 days *post mortem* at 2°C (20 to 30 N), whereas beef shear force values were around 100 N at the same time (Wiklund, Barnier, Smulders, Lundstrom & Malmfors, 1997). Rapid tenderness development in venison is caused by higher protease activity than in beef (Barnier, Wiklund, Vandijk, Smulders & Malmfors, 1999).

Researchers are continuously looking for ways to maximize beef production efficiency, including genetic benefits due to cross-breeding. Beef production in both tropical and subtropical regions utilize *Bos indicus* and *Bos taurus* cattle, primarily Brahman in the U.S in cross-breeding programs to improve cattle productivity by increasing disease and insect resistance, climatic tolerance, heterosis and additive genetic variation (Highfill, Esquivel-Font, Dikeman & Kropf, 2012). In terms of tenderness, Whipple, Koohmaraie, Dikeman, Crouse, Hunt and Klemm (1990) revealed that less tender *longissimus* muscle of *Bos indicus* cattle compared with *Bos taurus* cattle apparently was due to reduced *post mortem* proteolysis of myofibrillar proteins in *Bos indicus* cattle. This reduced proteolysis was associated with higher activity of calcium-dependent protease inhibitor (calpastatin) in *Bos indicus* cattle. Furthermore, at similar age, nutritional background, management and days on feed, carcasses from *Bos taurus* cattle are heavier, have more fat cover, larger ribeye area and greater intramuscular lipid percentage than *Bos indicus* and thus *Bos taurus*

carcasses which one factor that could positively influence tenderness (Highfill et al., 2012).

Calpastatin, a protease inhibitor, through its inhibitory action on calpains accounts for some differences in meat tenderisation. For example, toughness attributable to genetic differences correlates well with higher calpastatin activities (Koohmaraie, 1996). Similarly, the toughness often seen in animals with muscular hypertrophy, for instance, callipyge sheep or double-muscled Belgian blue cattle, tends to be associated with high levels of calpastatin activity (Fiems, Batjoens, Uytterhaegen, Moermans, Van Hoof & Boucque, 1993).

It has been suggested that increased meat toughness, linked with both genetic conditions and drug supplements, is largely due to alterations in protein synthesis and degradation in response to the calpain enzyme system (Kretchmar, Hathaway, Epley & Dayton, 1990).

If synthetic β -agonists can exert such effects on meat tenderness, it is important to understand the effects of the endogenous β -agonist adrenalin. For example, exercise during the immediate pre slaughter period has been shown to affect subsequent meat aging in sheep without affecting the ultimate pH (pHu) (Daly, Simmons & Devine, 1995). Adrenergic agonists stimulate pre slaughter glycogen usage and, if this continues, lead to an increase in pHu. In the intermediate pHu range between 5.8 and 6.2, meat is tough (Watanabe, Daly & Devine, 1996), but this toughness is attributed to a reduction in aging rate rather than a reduction in the final level of tenderness. Given enough aging time, meat in this range attains the same tenderness as meat of a normal pHu. However, reports have demonstrated toughness in meat that attained a normal pHu when β -agonists were administered pre slaughter (Fiems, Buts, Boucque, Demeyer & Cottyn, 1990); and Sensky, Parr, Bardsley and Buttery (1996) showed a reduced proteolytic potential in pigs treated in the *ante mortem* period with adrenaline, primarily due to a reduction in the calpain : calpastatin ratio. This supports the study of Daly, Simmons and Devine (1995) which showed that pre slaughter stress (thus high levels of circulating adrenalin) in combination with *post mortem* electrical stimulation, reduced the rate of tenderisation three times.

Clenbuterol significantly affected tenderness, although the effect was different for short-term and prolonged supplementation. Short-term supplementation increased toughness at

48 and 72 hours after slaughter, but neither initially (24 hours) nor finally (144 hours), indicating that the effect was to slow the rate of tenderisation (Simmons, Young, Dobbie, Singh, Thompson & Speck, 1997).

In contrast, prolonged clenbuterol treatment produced a significant increase in shear force value at all times *post mortem* and the pattern of shear force change was markedly different from that normally seen in lamb: the first shear force value, measured at 24 hours, was higher than is typical for unaged lamb *M. longissimus* based on findings from a previous experiment (Devine & Graafhuis, 1995).

In agreement with other studies, μ -calpain activity measured *in vitro* at slaughter was not influenced by either short-term or prolonged treatment with clenbuterol (Kretchmar et al., 1990; Wheeler & Koohmaraie, 1992). However, Simmons, Young, Dobbie, Singh, Thompson and Speck (1997) showed a significant difference occurred at 6 hours, when μ -calpain activities were lower in the treated animals, a difference that remained to 24 hours. Nevertheless, the inhibitor of calpain, calpastatin, was largely unaffected by short-term drug treatment, as well as m-calpain. Consequently, among these three activities, decreased μ -calpain activity indicated the most likely cause of the reduced rate of tenderisation. It was suggested that long-term clenbuterol-treated muscle had a sufficiently high μ -calpain activity at all times to tenderise the meat, but the high calpastatin activity bound the μ -calpain (an effect enhanced by the higher pHu in the treated animals), thus reducing proteolysis and tenderness development (Simmons et al., 1997).

The double-muscling in cattle seen in double-muscling Belgian blue cattle contains a low content of connective tissue, such as the *longissimus* muscle, the positive effect of double muscling on meat tenderness may be absent owing to a relatively more important contribution of myofibrillar and cytoskeletal protein degradation during the ageing process (Smet, 2004). Double-muscling cattle have consistently lower μ -calpain, calpastatin and cathepsin levels in the *M. longissimus* than for normal cattle, associated with changes in protein break down and in line with the reduced *in vivo* protein turnover. Results for other muscles on enzyme activities and *post mortem* proteolysis are scant. Both the changes in enzyme activities and protein concentration during tenderization observed in the *longissimus* muscle show that proteolysis occurs at a faster rate early *post mortem* in double-muscling animals, consistent with the more glycolytic muscle fibre type and the

earlier *rigor* development. However, total proteolysis and tenderization during full ageing seems to be lower in double-muscled animals. The overall effect on shear force value is variable, depending on the muscle studied and on the time/temperature treatment of the meat (Smet, 2004). Uytterhaegen, Claeys, Demeyer, Lippens, Fiems, Boucque, Van de Voorde and Bastiaens (1994) also reported that μ -calpain/calpastatin ratio is critically lower within double-muscled animals at 1 and 24 hours *post mortem*; they proposed a reduced μ -calpain activity in double-muscled animals. Further, it was suggested that calpastatin is still active and that is a sufficient calpastatin concentration in muscle to inhibit calpain activity.

Muscle type also affects ageing rate (Geesink, 1993). Ageing rate occurred faster in fast twitch glycolytic type II muscle (*longissimus dorsi*) than slow twitch oxidative type I (*triceps brachii*). Moreover, size and location of muscle might impact on tenderization rate. For that reason, location and size (*triceps brachii*) muscle led to slower temperature decline and also tenderization rate, but, tenderness value remained similar beyond 14 days of ageing (Geesink, 1993).

Tenderness development is also affected by storage time and temperature. Generally, the longer the time and higher the temperature the greater the tenderness, but at the same time realising that tenderness is limited by connective tissue. The question can be asked: why not age all meat to a tender endpoint? There are several reasons: hygiene cannot be maintained indefinitely, storage costs money, meat becomes over-tender and loose structure, and adverse flavours develop from fat oxidation and other reactions (Dransfield, 1994).

1.5.5. Intramuscular fat

Intramuscular fat is located in adipose cells that along with connective tissue comprise the endomysium and perimysium that encase muscle fibres and bundles of muscle fibres (Wood, 1990). The quantity of marbling (intramuscular fat) is considered as one of main quality attributes of meat (Wood, 1990). Subject to diet, intramuscular fat development increases as animals mature such that increased quantities of intramuscular fat coincide with older animals (Pethick, Harper & Oddy, 2004). Consumers in some countries are satisfied with intramuscular fat, notably the USA, while others, typically European, are not accepting (Ngapo & Dransfield, 2006).

Dikeman (1987) proposed that intramuscular fat content in meat might be responsible for 10-15% of palatability variation, but that could be culturally specific to the USA.

However, intramuscular fat plays a crucial role in providing flavour and juiciness that may extend to meat tenderness development (Webb & O'Neill, 2008). There are a few ideas to describe this association between intramuscular fat and tenderness. Many researchers have proposed that meat containing high quantities of fat tend to be more tender (Miller, 2004; Webb et al., 2008). It has been suggested that fat is less dense than heat-denatured protein, resulting in softer tissue overall and thus greater tenderness (Miller, 2004). Another explanation concerns the lubricating influence of fat. In general, fat is made up of triglycerides that stay within adipose cells. These fats are responsible for lubrication effect while meat is chewed, making chewing easier as muscle fibres and bundles slip past one another (Miller, 2004). Moreover, it is also explained that intramuscular fat acts as insulator towards heat-induced toughening resulting from cooking. Meat proteins bind water contained in muscle. When these proteins become denatured by heat, their capacity to bind water is greatly reduced. The more meat proteins are denatured, the more water is lost, leaving a resistant fibrous gel. Importantly, high quantities of fat within muscle provide a resistant layer that diminishes the heat transfer throughout the muscle (Miller, 2004). Moreover, it is proposed that the latent heat for melting fat would also result in less heat available to denature protein in muscle. Collectively meats containing a high quantity of fat will tend to be 'undercooked' resulting in more tender meat (Miller, 2004).

1.6. Strategies to improve meat tenderness

1.6.1. Cooking

Cooking affects meat tenderness for several reasons. First, intramuscular fat becomes softer because the melting point of fat is below that of cooking end points. However, fat is usually only a minor component of meat and the major determinant of cooked meat tenderness are the muscle proteins. In very general terms cooking makes connective tissue (collagen) softer and contractile proteins harder. Thus, while connective tissues are mainly responsible for toughness in raw meat, contractile proteins are mainly responsible for toughness (texture) for cooked meat. However, it is important to distinguish the effects of different temperature ranges as they affect connective tissues and contractile proteins (Taylor, 2003).

Meat proteins become denatured in accordance with three phases. First, myosin (rod and light protein chains) denatures at temperatures between 40 and 60°C. Second, sarcoplasmic proteins and collagen denature between 60 and 70°C and third actin denatures at about 80°C (Deng et al., 2002; Wright & Wilding, 1984). Denaturation of collagen is accompanied by contraction and fluid loss where the collagen is extensively cross-linked, as in older animals. This is a toughening effect and only extended heating can result in softening for meat from mature animals. Thus muscle with a high quantity of connective tissue (for example shank and chuck) requires extended wet cooking.

The denaturation of the contractile proteins, myosin and actin together present another complex picture. In the range of 60 to 77°C, contractile protein becomes harder and the meat tougher. Thus, cooked beef to internal temperatures of 60, 70 and 80°C generates shear force values of 4.6, 7.0 and 8.1 kgF respectively. These results show that the optimum temperatures to develop meat tenderness is 65°C (Herring & Rogers, 2003).

1.6.2. Electrical stimulation

Electrical stimulation was initially applied to inhibit muscle cold shortening by increasing glycolysis rate (Carse, 1973; Chrystall & Devine, 1975). This meant that carcasses entered *rigor* sooner and could be chilled or frozen sooner without suffering cold shortening. These phenomena also contributed to an increase in the rate of meat tenderisation (Savell, McKeith & Smith, 1981). Electrical stimulation has subsequently been widely used in meat plants to improve tenderness in beef, lamb, goat and venison (Chrystall & Devine, 1983; Chrystall & Hagyard, 1976; Davey, Gilbert & Carse, 1976; Drew, Crosbie, Forss, Manley & Pearse, 1988; Geesink, VanLaack, Barnier & Smulders, 1994), but not in pork. Even though electrical stimulation can increase tenderness in pork, it is not carried out commercially in pork because the problem of pale, soft and exudative (PSE) condition that arises from rapid glycolysis. *Post mortem* glycolysis is intrinsically more rapid in pigs than in ruminants (Rosenvold & Andersen, 2003) and this is compounded by the halothane-sensitivity gene that occurs in many breeds of pigs. This gene is responsible for very rapid rates of glycolysis that compounds the problem of low pH (from rapid glycolysis) coupled with high carcass temperature that is responsible for the PSE condition. Despite this phenomenon, some studies prove that electrical stimulation could be used successfully in pork with less reduction in water-holding capacity if carcasses are cooled effectively (Rosenvold et al., 2003), although this obviously carries a cost.

As an example of the effectiveness of electrical stimulation on tenderness, Wiklund, Stevenson-Barry, Duncan and Littlejohn (2001) found that meat from electrically stimulated venison carcasses were more tender than non-stimulated controls at 1 day, 7 days and 21 days *post mortem*. However, beyond 21 days the difference was negligible. In beef, Hopkinson, Ringkob and Bailey (1985) showed that shear force results of *M. longissimus* from electrically stimulated steer carcasses aged for 2 days were equivalent to those of *M. longissimus* from non-stimulated carcasses aged for 14 days. These results confirmed those of Savell, McKeith and Smith (1981), who found that shear force values of electrically stimulated beef samples at 1 day of ageing were similar to those of control samples aged for 8 days. Stimulation causes a temporary severe muscle contraction that can lead to supercontracture caused through localised excessive calcium ion release from the sarcoplasmic reticulum (Dutson, Smith, Savell & Carpenter, 1980; Ho, Stromer & Robson, 1996). It could be this extra calcium which causes the tenderisation to proceed, through early activation of calpain. At the same time, electrically stimulated carcasses are warmer in *rigor* than non-stimulated carcasses and higher temperatures will accelerate enzyme activity.

Because non-stimulated meat eventually becomes as tender as stimulated meat, the benefits of electrical stimulation to accelerate tenderization are restricted to meat that is frozen shortly after slaughter or sold to retail markets within a week of slaughter. Electrical stimulation has minimal benefit for chilled products held for many weeks, such as in the New Zealand chilled export lamb trade to the northern hemisphere. Stimulation causes no harm to tenderness but always carries a cost, and should only be applied where a net benefit can be shown (Hwang et al., 2003; Strasburg et al., 2007).

Another benefit of electrical stimulation in beef and lamb is the inhibition of *rigor* shortening that can occur at high temperatures. This finding was made by Devine et al. (2006) and Rosenvold et al. (2008). The latter group also showed that drip loss is not affected by electrical stimulation. The finding of electrical stimulation for beef and lamb could prevent *rigor* shortening is in direct contrast to experience with pork, where acceleration of glycolysis makes carcasses prone to the PSE condition as discussed earlier.

Electrical stimulation is always applied to whole carcasses or carcass sides rather than individual muscles. Moreover, there are many possible voltages, waveforms and times that

can be applied, and stimulation's impact on different muscles will be highly variable, particularly as each muscle or muscle group will have different responses arising from composition and morphology (Hwang et al., 2003). Existing and future technologies may allow stimulation of individual muscle groups, perhaps leading to more controlled tenderness outcomes.

1.6.3. Mechanical tenderisation

Mechanical tenderisation of meat can be viewed as a continuum between fine comminution in equipment such as a bowl chopper, mincing, blade or needle tenderisation (while still retaining the meat as a whole piece), and tenderisation by pressure, again with meat retained as a whole piece (Hopkins, 2004). Fine comminution and mincing are beyond the scope of this review because whole meat structure is lost, unlike when meat is tenderised by blades and needles (Maddock, 2008). Blade tenderisation involves minute cuts from a gang of blades or needles, either through a repetitive up-and-down motion applied industrially to meat on a conveyer (Figure 5a) or as a manual process (Figure 5b). Industrially, the method is applied to entire cuts such as whole ribeye rolls and shoulder clods. The Jaccard device (Figure 5b) was developed to tenderize meat by needles in homes or restaurants (Maddock, 2008). A variation of blade tenderisation is so-called 'cubing' where small blades on rollers cut the meat (Maddock, 2008). Muscle fibres are cut and crucially the connective tissues of muscle. As a result the meat needs less shearing in the mouth resulting in improved tenderness. Figure 6 shows the impact of mechanical tenderization on the tenderness of different beef cuts, the effect on human perception of tenderness improvement is significant but not marked. One inevitable consequence of blade/needle tenderisation is reduced hygiene. As with fully comminuted meat, blades and needles will distribute bacteria between cuts.

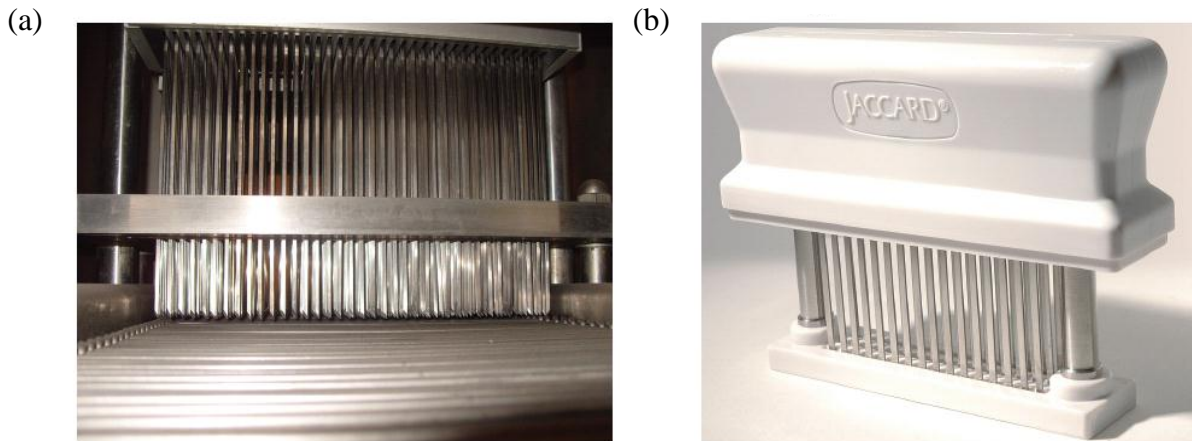


Figure 5. (a) Rows of needles or blades in a large mechanical tenderizer, (b) A hand-held tenderiser (Jaccard™).

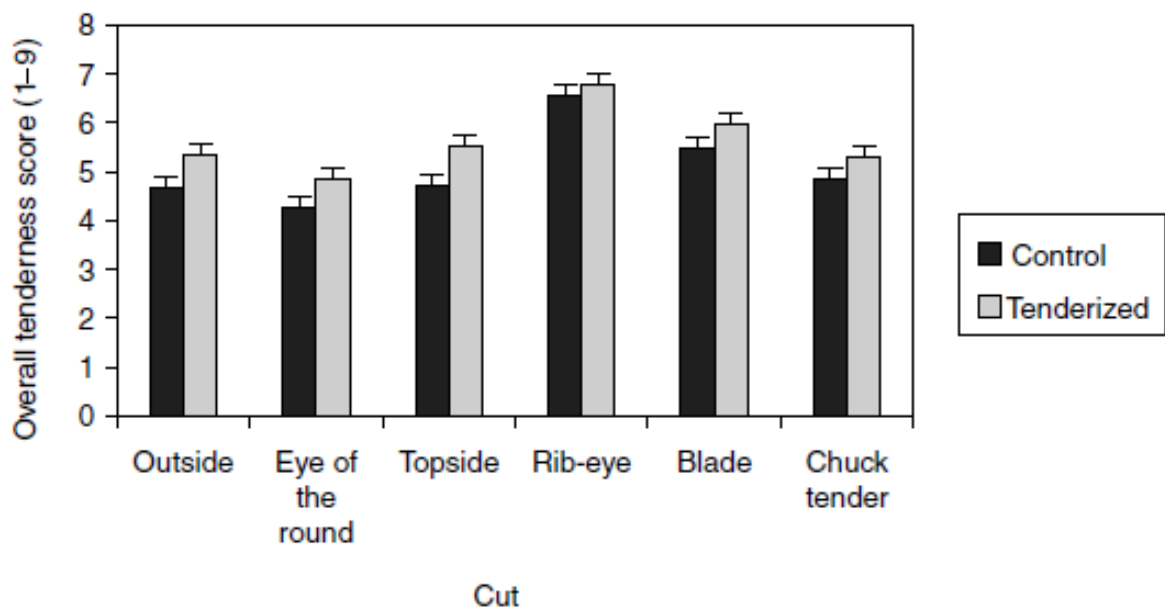


Figure 6. Impact of mechanical tenderisation on the tenderness of cuts from beef carcass (Jeremiah, Gibson & Cunningham, 1999).

Another form of mechanical tenderisation can be achieved by freeze/thaw (-20°C) that result in shrinkage of muscle fibres due to a dehydration of the cells and importantly increases the fragmentation of myofibrils (Hopkins, 2004). Ice occupies 9% more volume space than water, so freezing of meat tends to disrupt muscle cells (Foegeding, Lanier & Hultin, 1996). However, thawing of frozen meat always results in undesirable increased drip loss.

Ultrasonics is a new technique that might be applied to tenderise meat. Sound waves can

pass through materials at different speeds, including meat, and impart energy to the transmission material, which has the potential to cause physical disruption. This mechanism occurs particularly when the wavelength of ultrasonic soundwave is equivalent to the size of structural units of meat (Hopkins, 2004). Physical disruption of muscle contributes to the release Ca^{2+} from sarcoplasmic reticulum potentially resulting in acceleration of enzymatic activity. For instance, high intensity of ultrasound at 2.6 MHz (10 w.cm^{-2}) increase the release of cytosolic Ca^{2+} and increased sarcomere length by 10% in beef *M. semimembranosus*. Despite this effect, no clear advantage in reducing toughness has been reported (Hopkins, 2004). According to Hopkins, “the size of meat [pieces] and the use of muscles with a high connective tissue content could be some of the reasons for the apparent lack of effect of ultrasound on toughness”.

Another mechanical tenderization accounts for hydrodynamic pressure that has been introduced to diminish meat toughness due to a small quantity of explosives to form a shock wave that passes through water in divisions of milliseconds (Hopkins, 2004). This operation requires meat be held in container and surrounded by water because meat contains high amount of water. While the meat is placed in metal or plastic container, shock waves can be reflected from container wall and intersect. This phenomenon results in raising pressure to generate physical disruption of encapsulated meat. It is found that I-band of myofibrillar protein is broken down from Z-disk and fractions of A-band and I-band joining; tenderness is developed due to physical damage of muscle structure, particularly myofibrillar proteins (Hopkins, 2004). This method is applied to fresh meat with 70% improvement of meat tenderness effectively. This magnitude of improvement is sufficient for meat with high amount of connective tissue such as *M. semimembranosus*.

In another technology, hydrostatic pressure via a surrounding liquid is applied to meat to improve tenderness due to its high pressure and combined heat (Hopkins, 2004). Hydrostatic pressure is suitable for both conditions (*pre rigor* and *post rigor*) states of muscle that contributes to physical disruption of muscle structure. Both pressure and heat combination accelerates glycolysis rate leading to improvement in tenderness (Hopkins, 2004). *M. longissimus* performed with hydrostatic pressure showed the reduction in shear force values compared to control samples that both samples exposed to cold shortening. Moreover, this mechanism also results in myofibrillar degradation of proteins, in particular

of titin and actin depolymerisation; toughness induced-connective tissue also diminishes (Hopkins, 2004).

1.6.4. Exogenous enzymes

The most common method of meat tenderisation with exogenous enzymes is with serine protease enzymes that occur widely in plants. The generic name for these enzymes is papain that occurs in papaya leaves and fruit. This plant is widespread in the tropics, where it has been used for an indeterminate history to tenderise meat (Feiner, 2006; Warriss, 2000). Although papain-like from other plants, like pineapple, fig and kiwifruit, are very similar to papaya papain in terms of their amino acid sequences, they usually are referred to a unique name derived from the systematic plant name, thus ficin from fig, *Ficus*. Papain from papaya can be extracted to varying purities as a liquid or a powder from the latex of papaya leaves and fruit. Papain denatures around 80°C and its optimal pH for proteolysis of meat proteins is the same as the pH of meat, around 5.6. The amount of papain used with meat is critically important since higher amounts or long tenderisation time can result in a major loss of meat texture (Feiner, 2006; Warriss, 2000).

Ficin and bromelain (pineapple) are alternative enzymes that can be used, as can be an extract of ginger but these are rarely used (Belitz, Grosch & Schieberle, 2009; Weiner, 2006; Warriss, 2000), possibly because of price. Actinidin derived from kiwifruit (Arcus, 1959), and has been of particular interest to New Zealand. However, it is not commercially extracted for meat tenderisation, rather as dietary supplement to aid digestion of meat, marketed under the name Kiwi Crush (Vital Foods Ltd., Auckland). Interestingly its pH optimum is 4 on gelatine and myofibrillar proteins (Nishimaya, 2001), and this suggests the possibility of activity in the stomach which is notably acidic.

1.6.5. Tenderstretch

Tenderstretch refers to the method of hanging or suspending the animal carcass, in which an S-shaped hook is set in the eye or *obturator foramen* of the aitch bone to generate the hanging position different from that of conventional hanging through the Achilles tendon (Harris, 1974). When hung like this the hind legs are at roughly right angles to the vertical vertebral column (Figure 7). Tenderstretch is not applied during carcass dressing, which requires conventional Achilles hanging. Generally, Tenderstretch should be applied to carcass within 45 to 90 minutes of slaughter and while the carcass is in *pre rigor*. This

double handling adds cost that has to be recovered in a higher-priced product. If the angle of the hind leg is significantly less than 90°, it may indicate that Tenderstretch was applied after *rigor* development started and was thus applied too late.

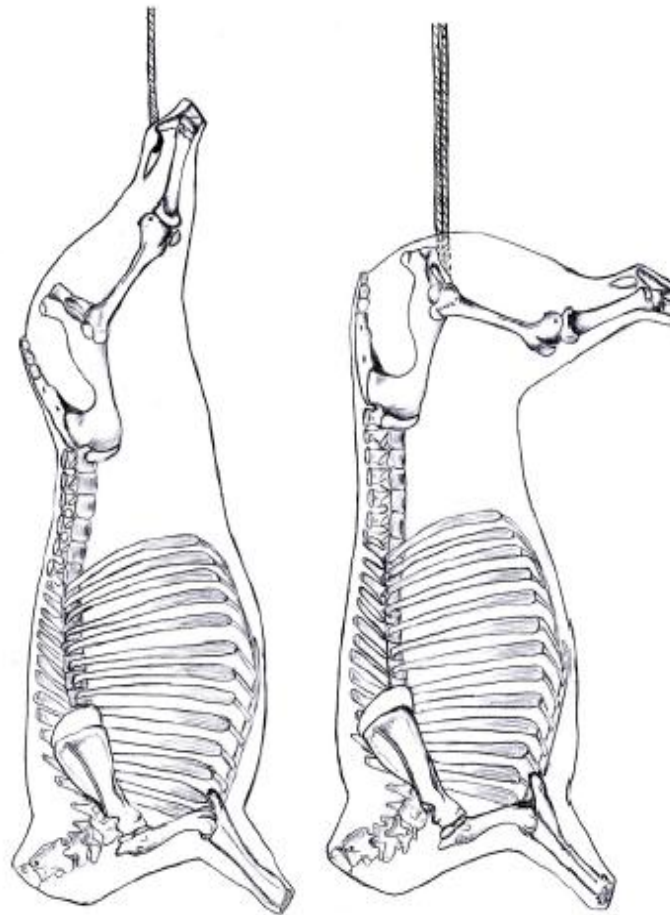


Figure 7. Schematic drawing of Tenderstretch. Carcass shapes resulting from suspension from the aitch bone (right), and the Achilles tendon (left) (Harris, 1974).

The basic idea of Tenderstretch is that *post rigor* muscles of the hind leg are not free to contract as they are when Achilles hanging is applied.

Many studies have explored the benefits of the Tenderstretch process for tenderness development within beef, lamb and pork. Herring, Cassens and Briskey (1965) showed that Tenderstretch hanging in beef caused longer sarcomere lengths, lower fibre diameters and improved tenderness for *longissimus*, *gluteus medius*, *biceps femoris* muscles. Similarly, Hostetler, Link, Landman and Fitzhugh (1972) showed effect of the Tenderstretch resulted in increased sarcomere length in beef *longissimus*, *semimembranosus* and *semitendinosus* muscles but not in *psoas major* muscle. Shear force values of two major muscles *longissimus* and *semimembranosus* decreased but were

unchanged in *semitendinosus* and *psoas major* muscles. The latter muscle, which is not a leg muscle, is always tender and any increase in tenderness might be counterproductive. The results with *M. semitendinosus* suggest that in muscles comprising larger quantities of connective tissue, that tissue is more important than the role of myofibrillar proteins in determining tenderness. Bouton, Harris, Shorthose and Baxter (1973b) showed that Tenderstretch increased tenderness in beef *longissimus*, *semimembranosus* and *gluteus medius* muscles. The shear force values of non-aged meat treated by Tenderstretch appeared the same as meat that had been aged for 21 days. However, they also showed that *semitendinosus* was unaffected by Tenderstretch. Joseph and Connolly (1976) confirmed all this work by reporting that Tenderstretch could improve tenderness within *longissimus*, *semimembranosus* and *gluteus medius* muscles. Nevertheless, tenderness values of *biceps femoris*, *semitendinosus* and *psoas major* muscles were not or partly improved by Tenderstretch. In general, Tenderstretch did not have the positive effect of reducing tenderness on the *semitendinosus* muscle. Joseph and Connolly (1976) also showed that Tenderstretch did not tenderise muscles of the forequarter of the carcass even if the forces acting through these muscles would be partially affected by Tenderstretch. At the same time it must be pointed out that forequarter muscles are, on average, richer in connective tissue than hind quarter muscles. In summary, Tenderstretch does not improve tenderness in muscles that are already very tender (*psoas major* muscles) and muscle where connective tissue toughness dominates (*semitendinosus* muscle).

Tenderstretch had also been applied to lamb, leading to improved tenderness of the *longissimus* muscle (Quarrier, Carpenter & Smith, 1972). Bouton, Harris, Shorthose and Baxter (1973b) showed that in mutton, Tenderstretch increased sarcomere length and the subsequent shear force values were reduced for *longissimus*, *semimembranosus*, *gluteus medius* and *biceps femoris* muscles compared with the Achilles hanging. Sarcomere length also increased in *M. semitendinosus* but the meat was not more tender, paralleling the results with beef, and presumably due to an abundance of connective tissue.

Generally, pork muscles are likely to be more tender than beef and lamb due to pork's rapid glycolysis and thus low possibility of cold shortening. Nonetheless, Tenderstretch applied to pork resulted in greater tenderness in the *longissimus* muscle when compared to unstretched muscles (Moller & Vestergaard, 1986). There had also been other supportive studies showing the positive effects of Tenderstretch in *longissimus* and *semimembranosus*

muscles of pork carcasses (Dransfield, Ledwith & Taylor, 1991; Taylor, Perry & Warkup, 1995).

1.6.6. Tendercut

A technique to stretch muscles as they enter *rigor* introduced by Wang, Claus and Marriott (1994) is Tendercut. In the Tendercut process, the carcass is hung by the traditional Achilles suspension method, and strategic cuts are made *pre rigor* through the bones, connective tissue, and fat such that the weight of the carcass below the cuts stretches the muscles. According to Taylor and Hopkins (2011b), Tendercut in its original form focussed on two areas of the carcass, severing between the 12th and 13th thoracic vertebrae to stretch the *M. longissimus lumborum*, and severing the ischium of the pelvis, the junction between the 4th and 5th sacral (tail) vertebrae and adjacent connective tissue, although small variations from carcass to carcass were not important in retaining the positive effects of Tendercut (Sørheim, Idland, Halvorsen, Frøystein, Lea & Hildrum, 2001). However, to ensure a sufficient stretching effect, obvious gaps must appear in the cutting areas (Claus, 2002). In the case of the cut between the 12th and 13th thoracic vertebrae, the *M. multifidus dorsi* is completely cut, but that muscle is minor compared with the *M. longissimus lumborum* (Figure 8). The Tendercut is commercially applied in at least the USA, Canada and Norway. Importantly, it does not require any capital expenditure, although there obviously is a labour cost in its application (Sorheim & Hildrum, 2002).

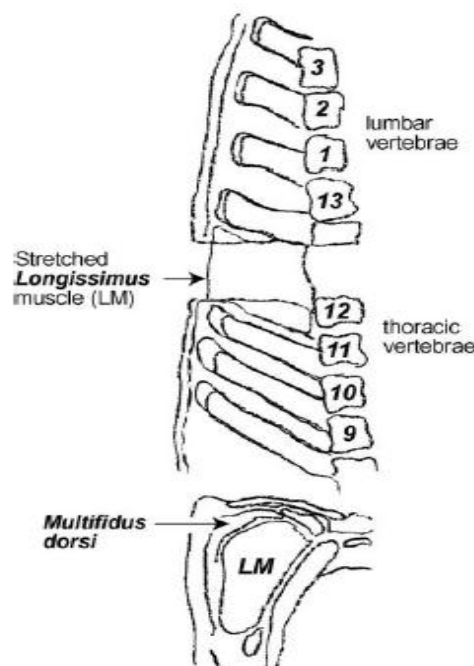


Figure 8. Schematic drawing of the Tendercut system with a cut in the 12th/13th vertebrae region of a beef carcass side (Claus, 2002).

A great deal of research has been done on the effects of the Tendercut on improving meat tenderness of loin and round muscles. Wang, Claus and Marriott (1994) investigated the effect of the Tendercut compared to conventional Achilles tendon suspension. Tendercut contributed to longer sarcomere length and a reduction of shear force values within beef round muscles (*vastus lateralis*, *rectus femoris*, and *vastus medialis*) compared with the Achilles hanging. Another study by Claus, Wang and Marriott (1997) showed that beef *longissimus*, *gluteus medius*, and *rectus femoris* muscles from Tendercut carcasses showed an increase in sarcomere length and a reduction in shear force value compared to untreated muscles. Similarly, in another study on four parts of *longissimus*, the shear force values were significantly decreased by the Tendercut treatment (Ludwig, Claus, Marriott, Johnson & Wang, 1997). However, when the Tendercut is applied to a light-weight heifer carcass that sarcomere length is increased in the loin and round muscles; however, tenderness is only improved in loin muscle (Beaty, Apple, Rakes & Kreider, 1999). The Tendercut also reduces variation in tenderness of beef *longissimus* muscles (Claus et al., 1997; Sørheim et al., 2001). The Tendercut is also effective to improve tenderness in pork *longissimus* muscle (Wang, Claus & Marriott, 1995).

1.6.7. Wrapping

Wrapping in cling polyethylene film is another mechanical technique that has been applied to *pre rigor* hot-boned muscle to improve tenderness by physically preventing shortening (Devine et al., 1999; Hildrum, Nilsen & Wahlgren, 2002; Rosenvold et al., 2008).

Wrapping contributes to decreasing time to attain satisfactory level of tenderness values (Rosenvold et al., 2008). The combination between wrapping and ageing contributes to significant reductions in shear force value and improved sensory results (Toohey, Hopkins & Lamb, 2008a). Wrapping costs money but meat storage usually involves packing of primal cuts by some plastic polymer. If that prevent shortening then costs can be turned to a commercial advantage.

Devine et al. (1999) showed that when *rigor* onset occurred in the high temperature range 20 to 35°C, wrapping reduced muscle shortening and consequently improved meat tenderness. In contrast, there was no effect of wrapping when *rigor* onset occurred at the normally 20°C. Results were similar when lamb *M. longissimus* was wrapped at 18 and 35°C (Devine, Payne, Peachey, Lowe, Ingram & Cook, 2002a). In another confirmative effect of wrapping, *pre rigor* beef *M. longissimus* was wrapped at 4°C. Wrapped muscles

were more tender than unwrapped controls (Hildrum, Andersen, Nilsen & Wahlgren, 2000). Nevertheless, in the same work, *longissimus* muscles chilled at 12°C and *semimembranosus* muscles chilled at 4 and 12°C showed no wrapping effect on tenderness. These contradictory results between muscles are probably due to the size and shape of the *semimembranosus* muscle that make it physically harder to reduce muscle contraction than in *longissimus* muscle.

The wrapping technique has been further developed in a system where *pre rigor* muscle is placed in expandable packaging, called Pi-Vac Elastropack (Meixner & Karnitzschky, 2001) (Figure 9). Pi-Vac system is processed by stretching tubes of elastic film in the interior of a packaging chamber. After muscle is inserted into the chamber, stretch is released and the film is reverted to its initial form. The elasticity of the film restricts diametrical muscle expansion because of longitudinal contraction of muscle. Wahlgren and Hildrum (2001) investigated the effect of Pi-Vac system on beef *longissimus* muscle at 4 and 14°C. Meat tenderness was improved by Pi-Vac at both temperatures, and it was thus possible to chill rapidly without loss of tenderness. Furthermore, Pi-Vac system generates muscle shapes that are purportedly more attractive (Hildrum et al., 2002). Pi-Vac system is currently a non-continuous system that involves a lot of manual labour and would have to be further modified to be adapted to a high-speed industrial processing line (Meixner et al., 2001).

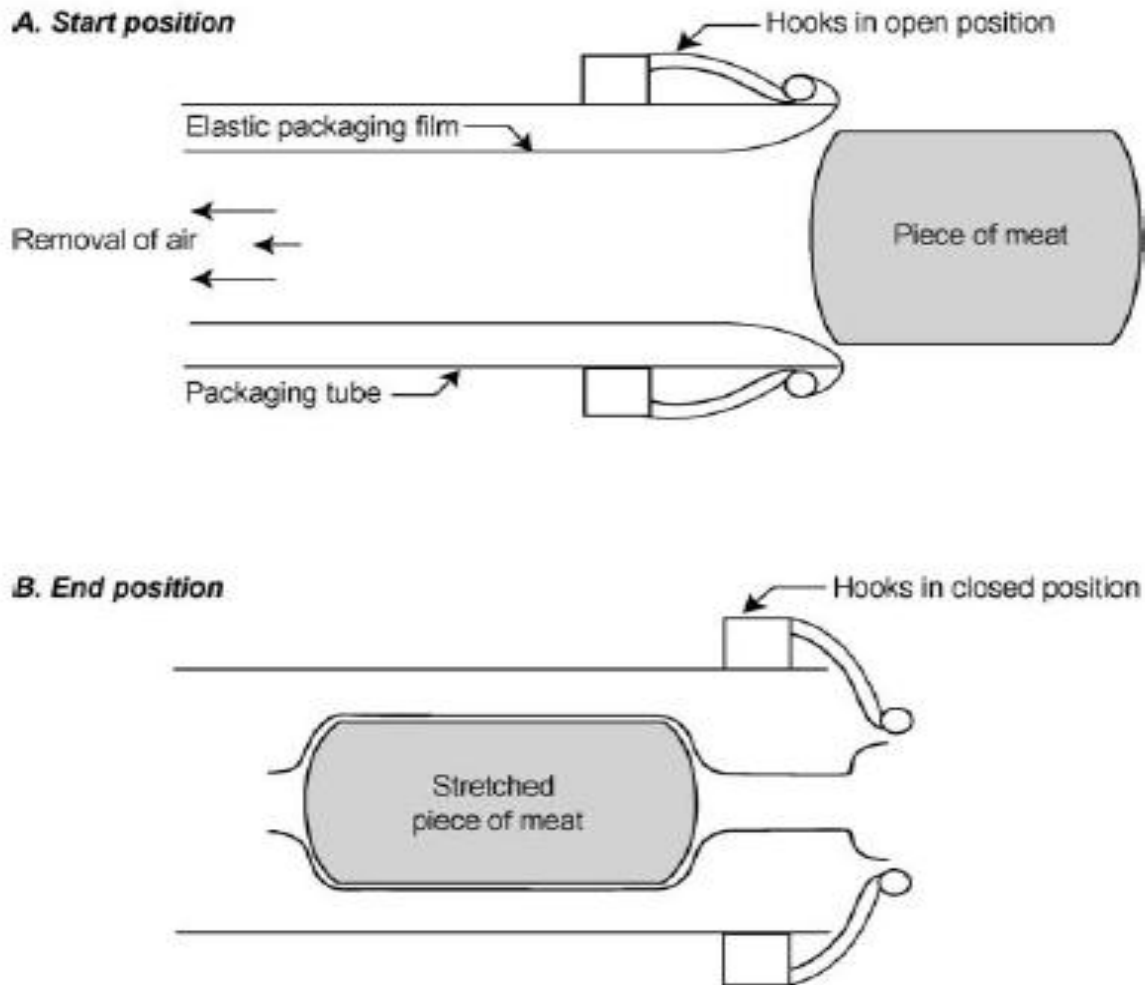


Figure 9. Schematic drawing of the Pi-Vac packaging system for hot-boned muscles; (A) start position and (B) after filling the machine (Meixner et al., 2001).

1.6.8. SmartStretch™/Smartshape™

The SmartStretch™/SmartShape™ (4S) system is a comparatively new technology based on air pressure applied to cold or hot-boned cuts in order to shape meat/muscle into a defined shape and at the same time to surround it with particular wrapping material to maintain that shape (Figure 10). The shape produced by the 4S involves a degree of muscle stretch that may stretch sarcomeres of muscle and also inhibit sarcomere contraction during *rigor* onset in order to improve meat tenderness (Pitt & Daly, 2010). These claims seem plausible.



Figure 10. SmartStretch™/SmartShape™ machine (Fix-All Services Company Ltd, Hamilton, New Zealand).

This machine system was developed in New Zealand (Simmons, Daly, Mudford, Richards, Jarvis & Pleiter, 2006) and then patented by Meat and Wool New Zealand Limited and Meat and Livestock Australia Limited in 2008 (Pitt et al., 2010). The ascribed marketing name was Boa, by reference to snakes that crush their prey. Subsequently the machine was developed to become SmartStretch™/SmartShape™ (4S). This improved machine can be applied to multiple meat cuts and can combine meat pieces into a defined shape (Simmons et al., 2006). The 4S system comprises an externally ribbed elastic sleeve that is surrounded by inflatable bladders in which air can be pumped into and out of. When air is pumped out of bladder compartments a negative pressure generates a bigger sleeve into which meat surrounded by a plastic wrapping (Figure 10) can be inserted. When air is pumped into the bladder compartment the pressure increases to compact the wrapped muscle/meat piece by a force perpendicular to the direction of the muscle fibres. Moreover, the mechanism also generates a peristaltic action that compresses the wrapped

muscle/meat towards the insertion end of elastic sleeve (Taylor et al., 2011b). This action amounts to stretching as schematically shown in Figure 11 (Pitt et al., 2010).

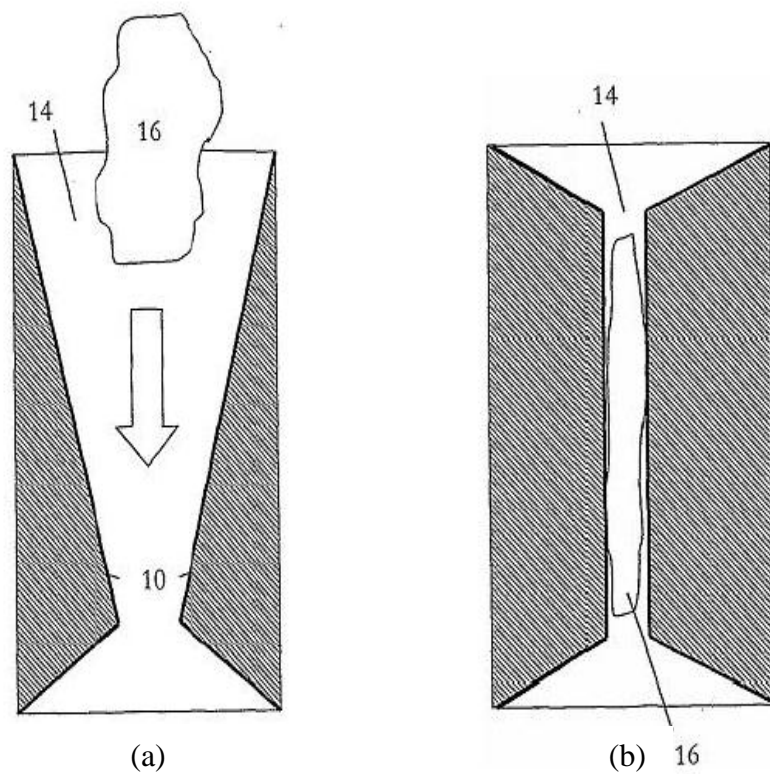


Figure 11. SmartStretch™/SmartShape™ concept. Insert meat (16) into the expanded sleeve (10) as shown (a) and apply compression causing the shaping and/or stretching of the meat (b) (Pitt et al., 2010).

There are two main claimed benefits of 4S treatment. First, (*post rigor*) meat cuts can be shaped and this is particularly useful in food service, where this an extended shape enhances portion control and decreases wastage. Second, 4S has the potential benefit to stretch meat *pre rigor* cuts to increase sarcomere length resulting in improved meat tenderness (Taylor et al., 2011b).

Current studies on the effects of 4S treatment on *pre rigor* sheepmeat and beef demonstrates critically varied results on meat quality. One experiment of 4S on *M. semimembranosus* of sheep muscle showed that the mean length of muscle increased by 24% resulting in a great increase in sarcomere length. Compared with untreated controls, shear force values decreased by 46% at 0 day *post mortem* and 38% after 5 days (storage at 4°C) (Toohey, Hopkins, Lamb, Nielsen & Gutzke, 2008b). This experimental result shows that 4S technology may have the capability substitute for conditioning by ageing. In a later

study, 4S was applied to an entire sheepmeat hind legs (Toohey, Ven, Thompson, Geesink & Hopkins, 2012). The mean length of leg increased by 14% and shear force values reduced by about 17% for *semimembranosus* and *biceps femoris* muscles at 0 day *post mortem*. However, there was no difference between treated and untreated legs at 5 days *post mortem*, in spite of increased sarcomere length.

4S studies on beef shows different results in beef muscles including *semimembranosus*, *longissimus lumborum* and *gluteus medius*. Studies by Simmons, Cairney, Auld, Nagle and Mudford (1999) and Toohey, Kerr, van de Ven and Hopkins (2010) found that these stretched beef muscles had no effect on tenderness in particular percentages of increased length. In these studies, the stretch engendered by 4S ranged between 10 and 52% (Toohey et al., 2010). To cite a specific experiment, Taylor, Hopkins and van de Ven (2010) showed that a 22% increase in the length of the *M. semimembranosus* had no impact on shear force or sensory values, although the beef samples used in these experiments were from older animals where panellists would have difficulty distinguishing between tough and extremely tough meat (Taylor, Toohey, Ven & Hopkins, 2012; Toohey et al., 2012). However, there was a reduction in tenderness variability due to the 4S treatment. This latter work was done in Australia where hot boning of beef is applied to aged cows and bulls, where background toughness is due to mature connective tissue may dominate perceptions of toughness (Taylor et al., 2011b).

1.7. Objective of this thesis

Since only a few studies have determined the efficacy of 4S on meat tenderness, there is still limited information regarding to effects of 4S treatment on beef tenderness development, water-holding capacity and myofibrillar protein degradation of beef muscles. Therefore, the objective of this thesis is to examine the effect of *pre rigor* stretching by 4S on sarcomere length, shear force, water-holding capacity and proteolysis of *M. longissimus dorsi* sourced from steers aged around 2 years.

Chapter 2

Materials and Methods

2.1. Materials and equipment

2.1.1. Muscles

A total of 12 steers (approximately 2 year-old of age with mean hot-carcass weight of 227 kg) were slaughtered at the Ruakura abattoir (Hamilton, New Zealand), using the standard slaughter procedure. Left and right sides of *M. longissimus dorsi thoracicus* were taken and randomly allocated for 4S treatment (4S) or without 4S treatment (control).

2.1.2. Chemicals and solutions

Sodium dihydrogen orthophosphate mono hydrate ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$), disodium hydrogen phosphate heptahydrate ($\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$), sodium dodecyl sulfate ($\text{CH}_3(\text{CH}_2)_{11}\text{OSO}_3\text{Na}$) and glycerol ($\text{C}_3\text{H}_8\text{O}_3$) were purchased from BDH AnalaR Laboratory Supplies (Poole, England). Tris [Tris-(hydromethyl)-aminomethane] ($\text{C}_4\text{H}_{11}\text{NO}_3$) was bought from Applichem (Damstadt, Germany). The Sigma Chemical Company (St. Louis, MO, USA) supplied disodium ethylenediamine tetraacetate (EDTA) ($\text{C}_{10}\text{H}_{14}\text{N}_2\text{O}_8\text{Na}_2 \cdot 2\text{H}_2\text{O}$) and pyronin Y dye. Disodium hydrogen orthophosphate di hydrate ($\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$) was from BDH. Sodium chloride (NaCl) was obtained from LabserveTM Biolab (Australia). Glycine ($\text{NH}_2\text{CH}_2\text{COOH}$) and methanol (CH_3OH) were sourced from Fisher Scientific (Leicestershire, UK) and glacial acetic acid was from J.T. Baker Chemical Company (Phillipsburg, NJ, USA). Coomassie Brilliant Blue R-250 was purchased from BIO-RAD Laboratories (California, USA). A molecular weight marker (marker) was used as Precision Plus ProteinTM KaleidoscopeTM standard, sourced from BIO-RAD Laboratories (California, USA). BIO-RAD Laboratories also supplied Bio-Rad DCTM protein assay reagent S and Bio-Rad DC protein assay reagent A. Pierce ECR Western blotting substrate was purchased from Thermo Scientific (Illinois, USA).

2.1.3. Equipment

The SmartStretchTM/Smart ShapeTM machine (designated as the 4S machine) was supplied by Fix-All Services Company Ltd, Hamilton, New Zealand (Figure 10). The 4S machine simultaneously stretches and wraps muscle samples excised *pre rigor* from beef carcasses. The pH meter was a Testo type 206 (Testo AG, Lenzkirk, Germany) fitted with type 206

pH electrode and a thermocouple probe to measure pH and temperature simultaneously. An Ika[®]-Labortechnik (Staufen, Germany) Ultra-Turrax fitted with a T25 dispersing element was used to disperse meat samples for sarcomere length measurement. The water bath for cooking meat sample was a custom model heated by a 3-phase resistance coil and could maintain around 100 L at a rolling boil (Figure 12).



Figure 12. The water bath used to cook meat samples. Weighted barrier bags contained the meat sample with a thermal probe inserted to the core, with its wire leading to a data logger.

The transmission light microscope (Orthoplan, Germany) was fitted with a 100X objective oil immersion lens. Light could be directed to a camera that fed data to a computer program Pro Plus V 4.5 (Orthoplan) to measure sarcomere length of dispersed meat samples (Figure 13).



Figure 13. Microscope for sarcomere length measurement.

Shear force was measured by two tenderometers, the G2 portable tenderometer made and supplied by Fix-All Services Company Ltd. and the MIRINZ tenderometer (Figure 14). The MIRINZ tenderometer was custom-built by the former Meat Industry Research Institute (Inc.) and has been used for decades. The MIRINZ standard shear force results appeared in kPa whereas the G2 is kgF. The data can be interconverted by a developed equation that converts shear force from MIRINZ tenderometer kPa to kgF.



Figure 14. (a) G2 tenderometer

(b) MIRINZ tenderometer

2.2. Methods

2.2.1. Animals and treatments

Twelve steers were slaughtered at the abattoir, using a standard humane slaughter procedure: the animals were electrically stunned, slaughtered by throat cut and then electrically stimulated around 30 seconds *post mortem* using low voltage electrical stimulation (80V peak, 14.28 pulse s⁻¹ for 30 seconds). The time of slaughter was recorded. Both sides of *M. longissimus dorsi thoracicus* were excised from the carcass and allocated randomly within animal to either the 4S treatment or the control (see Appendix 1 for the experimental design). 4S replicates were immediately processed by the 4S machine and packaged into thick polyethylene cling film (Figure 10) while control replicates were wrapped in polyethylene cling film. The length of muscle before and after 4S treatment was measured. All muscles were transported to Meat Science and Technology at Agresearch Centre, Ruakura (5 minutes transit) and stored at - 1.5°C for 24 hours. At 1 day *post mortem*, the length of 4S loins was remeasured and pH was determined on all loins. All meat pieces were trisected laterally to generate the subplot ageing times (1, 7 and 14 days, again according to a randomisation design (Appendix 1). Day 1 sections which were laterally cut into five subsamples that were randomly destined for (cooked) shear force (MIRINZ) and (G2), drip loss, proteolysis and sarcomere length measurements. Day 7 and day 14 pieces, 48 in all, were weighed, vacuum packed in barrier bags and held at - 1.5°C. On 7 and 14 days of ageing pieces were removed from bags, patted dry and reweighed to calculate purge loss. pH was remeasured, and the pieces were cut into five subsamples as described above. On day 14, the remaining 24 pieces were similarly treated.

In summary, *M. longissimus dorsi thoracicus* from two sides of 12 steers generated meat from three days of storage, from which five subsamples were cut for further measurements (2 x 12 x 3 x 5 = 360 pieces)

2.2.2. Measurements

2.2.2.1. Purge loss

Purge loss on vacuum storage at - 1.5°C could be calculated on 7 and 14 day aged meat pieces before subsampling. The pieces were removed from the vacuum bags, blotted dry with paper towels and reweighed (final weight) to measure purge loss. Purge loss was calculated as:

$$\text{Purge loss (\%)} = 100 \times [(\text{Initial weight} - \text{Final weight}) / \text{Initial weight}]$$

2.2.2.2. pH

pH was determined on 1 , 7 and 14 days *post mortem* meat samples. The Testo 206 pH meter was calibrated at 25°C with a pH 4 and a pH 7 buffer solution (Mallinckrodt Chemicals, USA).

2.2.2.3. Drip loss

Drip loss was measured on 1, 7 and 14 days of ageing that method was described by Honikel bag method (Honikel, 1998). Each subsample (approximately 60 g) was trimmed free of subcutaneous and seam fat and any visible connective tissue. Subsample was then weighed and placed in a single layer of onion bag netting that was twisted closely at the top and suspended inside a sealable, water-impermeable container with hook in the lid, in which meat did not contact the container walls. The assemblies were held at 4°C for 48 hours. The meat was then recovered, blotted dry and reweighed. Drip loss was calculated as:

$$\text{Drip loss (\%)} = 100 \times [(\text{Initial weight} - \text{Final weight}) / \text{Initial weight}]$$

2.2.2.4. Cooking loss and shear force

Meat pieces were weighted, placed in barrier plastic bags and cooked from frozen in the 99°C water bath maintained at a steady boil until the internal temperature 75°C of each was reached. Temperature of the thermocouples inserted to the centre of each piece was monitored by a Digi-Sense scanning temperature logger (Eutech Instruments Pte. Ltd., Singapore). When each piece attained 75°C the thermocouple was withdrawn and the bags placed in ice slurry for overnight storage at 1°C. As required for testing, pieces were removed from the bags, patted dry with paper towels and reweighed. Percentage of total cooking loss was calculated as:

$$\text{Cooking loss (\%)} = 100 \times [(\text{Initial weight} - \text{Final weight}) / \text{Initial weight}]$$

Subsequently, 10 × 10 mm cross section strips (10 replicates for each piece) were excised parallel to the fibre direction, which were sheared by the MIRINZ tenderometer perpendicular to the fibre direction (Macfarlane and Marer (1966); Figure 14), as one subplot and with the G2 tenderometer as the other (Figure 14).

2.2.2.5. Sarcomere length

The meat destined for the determination was cut to generate 1 g subsamples that were dispersed in 10 mL of 0.25 M sucrose solution for 15 seconds with Ultra-Turrax T25 homogenizer (Ika® - Labortechnik, Germany) at full speed. A single drop of the resulting dispersant was placed on microscope slide, covered with a glass cover slid examined photographed for image analysis as described by Rodbotten, Lea and Hildrum (2001). For each subplot 10 pictures were taken where there were at least 10 sarcomeres present in each field of view to calculate mean sarcomere length value (µm).

2.2.2.6. Western blot to determine desmin degradation

Protein solubilisation

Animal numbers 1, 3 and 10 at 1 and 14 days of ageing from both treatments were arbitrarily selected for the determination of desmin, a useful marker protein for *post mortem* proteolysis. These meat pieces were frozen with nitrogen liquid and then powdered with a blender. Briefly, 1 g of each subsample was suspended in 10 mL of solubilising buffer (10 mM sodium dihydrogen orthophosphate mono hydrate, 10 mM disodium hydrogen phosphate hepta hydrate, 2% w/v SDS, pH 7.0). Samples were dispersed using a Teflon-tipped Potter-Elvehjem tissue grinder for 20 seconds and the mixtures poured into 50 mL conical tubes. These were centrifuged at 1500 gravities for 15 minutes at 25°C and supernatant recovered.

Determination of protein concentration

Protein concentration was determined according to the method of Lowry, Rosenbrough, Farr and Randall (1951) using the BIO-RAD kit. Each supernatant was diluted 1:20 (10 µL protein sample and 190 µL DDI-H₂O) in duplicate and vertexed. Detergent Compatible (DC) Protein Assay was performed by adding 5 µL of DDI-H₂O into wells and 5 µL of BSA protein assay standard (Thermo Scientific Pierce, USA) with different concentrations (0.184; 0.368; 0.735 and 1.47 mg/ml) into various wells and 5 µl of each protein samples into other wells on the plate as shown in Figure 15. After reagent A' was made from 20 µl of solution S (BIO-RAD DCTM protein assay reagent S) and 1 ml of solution A (BIO-RAD DCTM protein assay reagent A, Hercules, CA); 25 µl of reagent A' was put in each wells and then 200 µl of reagent B (BIO-RAD DCTM protein assay reagent B) was inserted. This plate was left for 15 minutes and then taken to check the desired concentration of protein

from supernatant by computer software. The layout of the 96-well plates used for this work was summarised in Figure 15.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		H ₂ O	H ₂ O	Standard 0.18mg/ml	Standard 0.18mg/ml	Standard 0.36mg/ml	Standard 0.36mg/ml	Standard 0.74mg/ml	Standard 0.74mg/ml	Standard 1.47mg/ml	Standard 1.47mg/ml	
C		H ₂ O	H ₂ O	Standard 0.18mg/ml	Standard 0.18mg/ml	Standard 0.36mg/ml	Standard 0.36mg/ml	Standard 0.74mg/ml	Standard 0.74mg/ml	Standard 1.47mg/ml	Standard 1.47mg/ml	
D												
E	CON 101	4S 101	CON 1401	4S 1401	CON 103	4S 103	CON 1403	4S 1403	CON 1010	4S 1010	CON 14010	4S 14010
F	CON 101	4S 101	CON 1401	4S 1401	CON 103	4S 103	CON 1403	4S 1403	CON 1010	4S 1010	CON 14010	4S 14010
G	CON 101	4S 101	CON 1401	4S 1401	CON 103	4S 103	CON 1403	4S 1403	CON 1010	4S 1010	CON 14010	4S 14010
H	CON 101	4S 101	CON 1401	4S 1401	CON 103	4S 103	CON 1403	4S 1403	CON 1010	4S 1010	CON 14010	4S 14010

Figure 15. Layout of 96-well to determine protein concentration, CON 101= Control, animal #1 and 1 day *post mortem*; 4S 101= Stretching, animal #1 and 1 day *post mortem*.

Dilution

After determining the protein concentration, the volume of protein samples was made by proportional volume of protein supernatant and solubilising buffer to obtain 1 mL final volume. Wang's solution comprised of 30 mM Tris, 3 mM EDTA, 3% w/v SDS, 30% v/v glycerol, 0.003% w/v pyronin Y, pH 8 (0.5 mL) plus 0.1 mL mercaptoethanol were added to above protein solution. This solution was calculated to yield a final concentration of 4 mg. mL⁻¹ and a total volume of 1.6 mL. This mixture was immediately heated in a heating block for 15 minutes at 50°C.

Protein concentration check

To assess protein concentration for proper dilution, 15 % Tris-HCl gel (BIO-RAD Laboratories) was used. 7 μ L molecular weight standard was pipetted and inserted into well for standard protein to check protein extract sample and 10 μ L of each protein extract sample was put into the wells overlaid with running buffer (25 mM Tris, 192 mM glycine, 2 mM EDTA and 0.1% w/v SDS). Electrophoresis was run at 120 V potential for 2 hours in a BIO-RAD Criterion Cell system. The extracted gel was subsequently stained with 0.1% Coomassie Blue stain solution (Coomassie Blue destain solution plus 0.1 % Coomassie Brilliant Blue) for 90 minutes. Then this gel was destained with Coomassie Blue destain solution (40% v/v methanol, 7% acetic acid) twice for 2 hours each time. The resulting gel image was captured to compare protein density bands with molecular weight standard of proteins.

Protein gel for transfer and transfer to membrane

After the extracted protein solution was checked accurately, 15 % Tris-HCl gel was used for protein gel to transfer to membrane. 7 μ L molecular weight standard was pipetted and inserted into well for standard protein and 10 μ L of each sample was put into the wells on gel with running buffer (25 mM Tris, 192 mM glycine, 2 mM EDTA and 0.1% w/v SDS). Then gel was run in the BIO-RAD Criterion Cell system at 120 V potential for 2 hours. After electrophoresis, the gel was placed in transfer buffer solution (25 mM Tris, 192 mM glycine, and 15% v/v methanol) in order to prepare cassette. Polyvinylidene fluoride (PVDF) membrane, sponge, cassettes and methanol box were prepared in advance. Cassette was placed in BIO-RAD transfer tank within transfer buffer solution. To make sure that molecular weight standard and the “ear notch” were both on the right side and cassette placed in a transfer tank with similar direction (black at the back) and run at 90 V potential for 90 minutes. This process was shown in Figure 16.

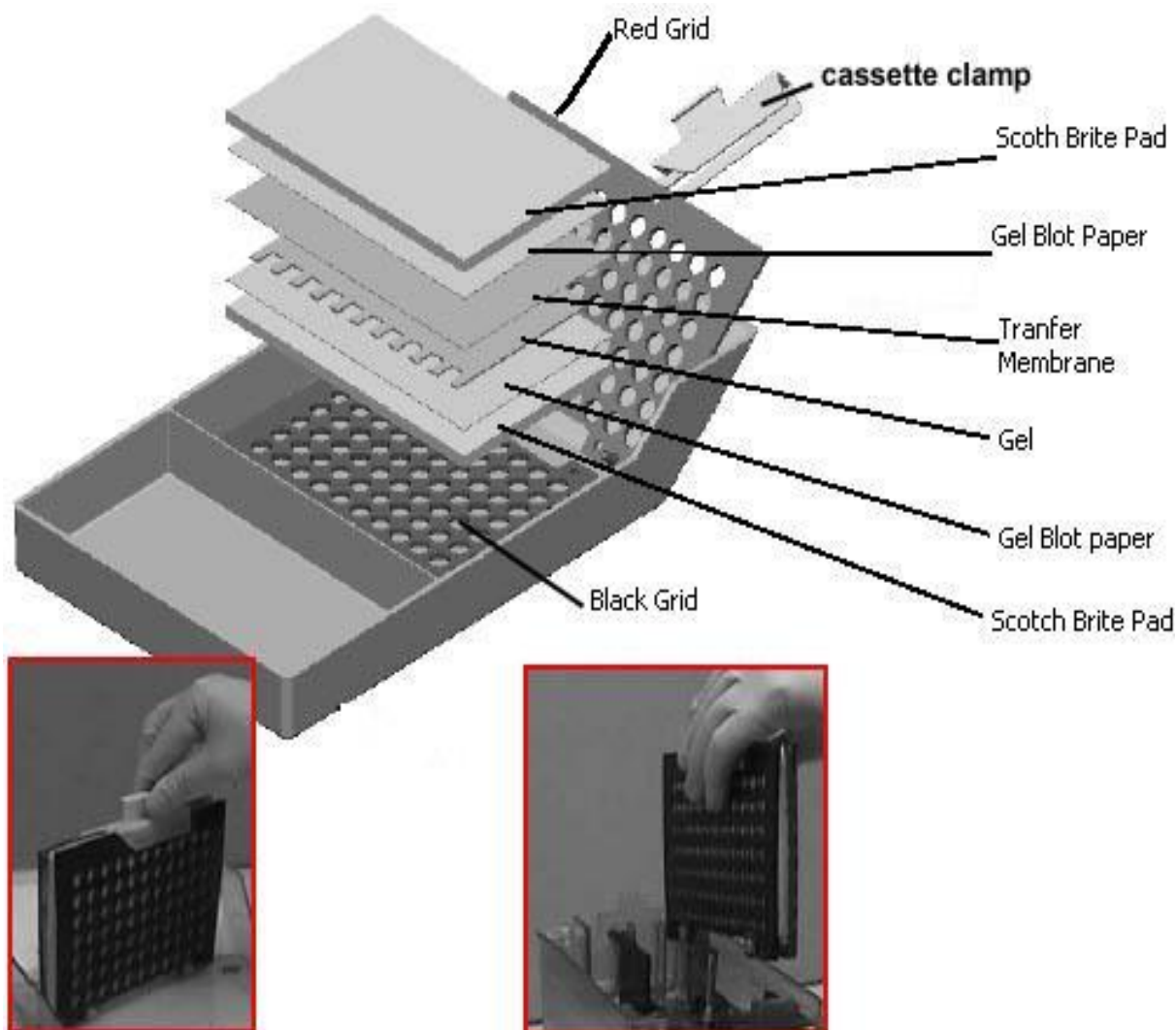


Figure 16. Transfer of protein to membrane by Western blotting, showing the arrangement of the blotting 'sandwich'.

Western blotting

Western blotting for desmin degradation was performed as described by Kim, Huff-Lonergan, Sebranek, and Lonergan (2010). Whole muscle protein sample preparation for the blot was conducted by following the procedure of Lonergan, Huff-Lonergan, Rowe, Kuhlers, and Jungst (2001). Following transfer, the membrane was blocked in blocking solution to binds to any remaining protein binding sites after primary antigen or antibody was bound [PBS, 1% v/v Tween 20 (PBST) and 5% of non-fat milk] for overnight at 4°C. The blot was washed three times, 10 minutes per wash, in PBST. The prepared gel samples were loaded at 40 µg of protein per well and the same amount of internal reference was loaded onto the gel. The blot was incubated at 4°C with the primary antibody (desmin = 1 : 20,000 dilution with PBS-Tween, monoclonal anti-desmin antibody, D1033; Sigma, St.

Louis, MO, USA) and washed three times (10 minutes each) with PBS tween. Its respective secondary antibody (desmin= 1 : 20,000 dilution with PBS-Tween, goat anti-mouse linked to horse radish peroxidase, No. 170-6516, BIO-RAD Laboratories, Auckland, New Zealand) was added and washed there times (10 minutes each) with PBS tween. Protein bands were detected using a chemiluminescent detection kit (Pierce ECR Western Blotting Substrate, LF 144464A, Rockford, IL, USA) and photographed in a G-Box: Chem HR 16 (Syngene, USA). Degradation product of desmin was expressed as a ratio compared to the internal reference in which the internal reference was 14 days *post mortem* beef bovine.

2.2.2.7. Data analysis

The experimental data were analyzed as a split-plot design, where each animal served as the whole-plot portion to which the stretching treatments [control (CON) or SmartStretchTM/SmartShapeTM (4S)] were randomly assigned to each side of a carcass [n=12; (12 cattle x 2 sides per cattle)/2 treatments]. In the subplot, three subsamples from each loin were assigned to different ageing periods. The data were analyzed by the PROC Mixed procedure of SAS for ANOVA (SAS, 2007). Type-3 tests of fixed effects for ageing time and random effects for animal and animal by stretching were determined. Least square means for all traits of interest were separated (F test, $P < 0.05$) by using least significant differences, using the *P* diff option.

Chapter 3

Results and Discussion

3.1. pH, loin length and sarcomere length

4S did not affect the pH of loins throughout the ageing period ($P > 0.05$; Table 1).

Immediately after the stretching, the 4S loins had an increased length compared with the controls, 33 ± 9 % (mean \pm sd). 4S maintained the stretched loins into consistent shape.

This length maintained at 32 ± 12 % (mean \pm sd) at 1 day *post mortem* (Figure 17).

However, sarcomere length was not influenced ($P > 0.05$) by the 4S treatment, with a mean sarcomere length of $1.9 \mu\text{m}$ (Table 1). Taylor, Toohey and Hopkins (2011a) found similar results from hot-boned beef strip loins (*M. longissimus lumborum*) in which sarcomere length was not different between stretched and control muscles accounting for $1.7 \mu\text{m}$ and $1.8 \mu\text{m}$ ($P > 0.05$), regardless of the 16.8 % increase in length by 4S. However, in hot-boned beef *M. semimembranosus*, they found an increase in muscle length by 40.5% accompanied with a significant increase in sarcomere length by a 4S treatment, although this result did not influence ($P > 0.05$) shear force values (Taylor et al., 2011a). In contrast to those beef studies, a positive impact of 4S on sarcomere length and tenderness improvement in sheep meat was reported. A 24% increase in length with a 24% decrease in circumference of sheep *M. semimembranosus* after 4S resulted in a significant increase in sarcomere length (2.19 and $1.54 \mu\text{m}$ for 4S and control, respectively) and subsequent shear force reductions of 46% at 0 day of ageing and 38% at 5 days of ageing (Toohey et al., 2008b). In their continuous study of applying 4S to a whole sheepmeat hind leg, a significant increase in sarcomere length of *M. semimembranosus* (1.82 and $1.61 \mu\text{m}$ for 4S and control, respectively) along with a 14% increase in length and subsequent reductions in shear force values were also reported (Toohey et al., 2012). If the beef muscle becomes 32%, 17% or 40% longer when using 4S and sarcomeres are unaffected which structure in the muscle is then stretched. More speculation, discussion and possible explanation to this phenomenon would be desirable. However, taking into account these differences, it is suggested that the impact of 4S on sarcomere length varies by muscles and species. Sheep muscles appear to respond to 4S treatment more than beef muscles in respect of sarcomere length. Further, the result from present study found that 4S did not affect sarcomere length of *pre rigor* beef muscle with the given increase in muscle length, which indirectly indicate that the current 4S technology may not sufficiently attribute to reduce the degree

of overlapping actin and myosin (Hopkins & Thompson, 2001) and/or a physical disruption of the Z-disk proteins such as actin, titin and nebulin (Hopkins, Garlicks & Thompson, 2000).

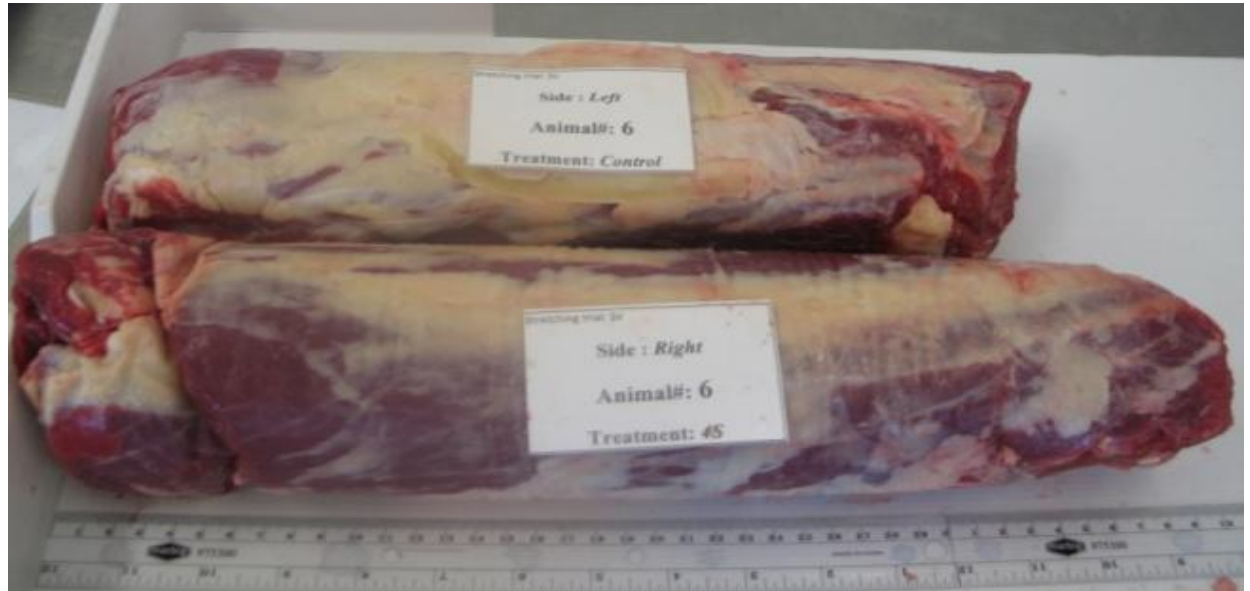


Figure 17. A representative photo of control (above) and stretched loins (below).

Table 1. Means of pH, purge loss, drip loss, cooking loss and sarcomere length at different *post mortem* days (1, 7 and 14 days) for SmartStretchTM/SmartStretchTM(4S) and control (CON) loins.

Trait	Day 1		Day 7		Day 14		SE [†]	P-value	
	CON	4S	CON	4S	CON	4S		Treat-ment	Day
pH	5.6	5.6	5.5	5.6	5.7	5.6	0.02	0.78	0.001
Purge loss (%) [*]			1.2	1.4	1.8	2.1	0.20	0.12	≤0.001
Drip loss (%)	1.9	1.7	3.7	2.9	2.8	2.8	0.40	0.27	≤0.001
Cooking loss (%)	20.8	21.2	21.1	21.1	20.5	20.5	1.24	0.85	0.81
Sarcomere length (μm)	1.9	1.9	1.9	2.0	1.9	1.9	0.04	0.19	0.04

* Purge loss was measured only at 7 and 14 day *post mortem*.

[†] SE is the pooled standard error.

Figure 18 combined the sarcomere length and different percentage increase in length of stretched muscles.

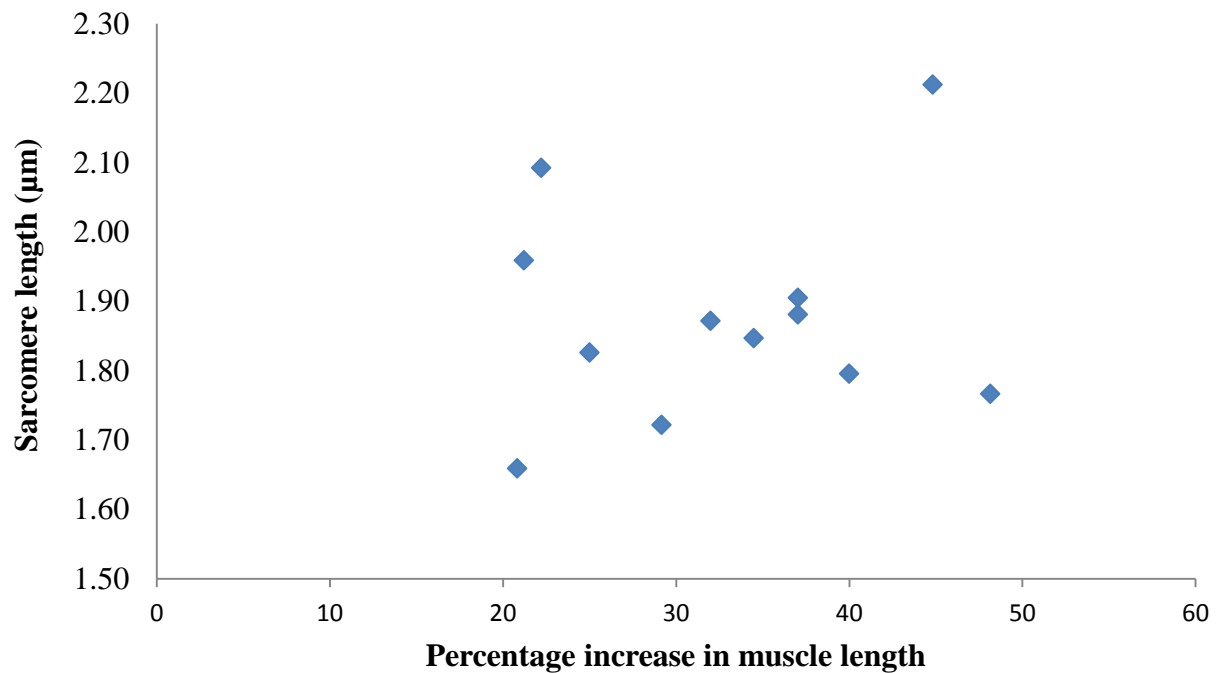


Figure 18. Relationship between sarcomere length (μm) and the percentage increase in length. A linear equation fitted to these data had a low correlation coefficient, $r = 0.138$.

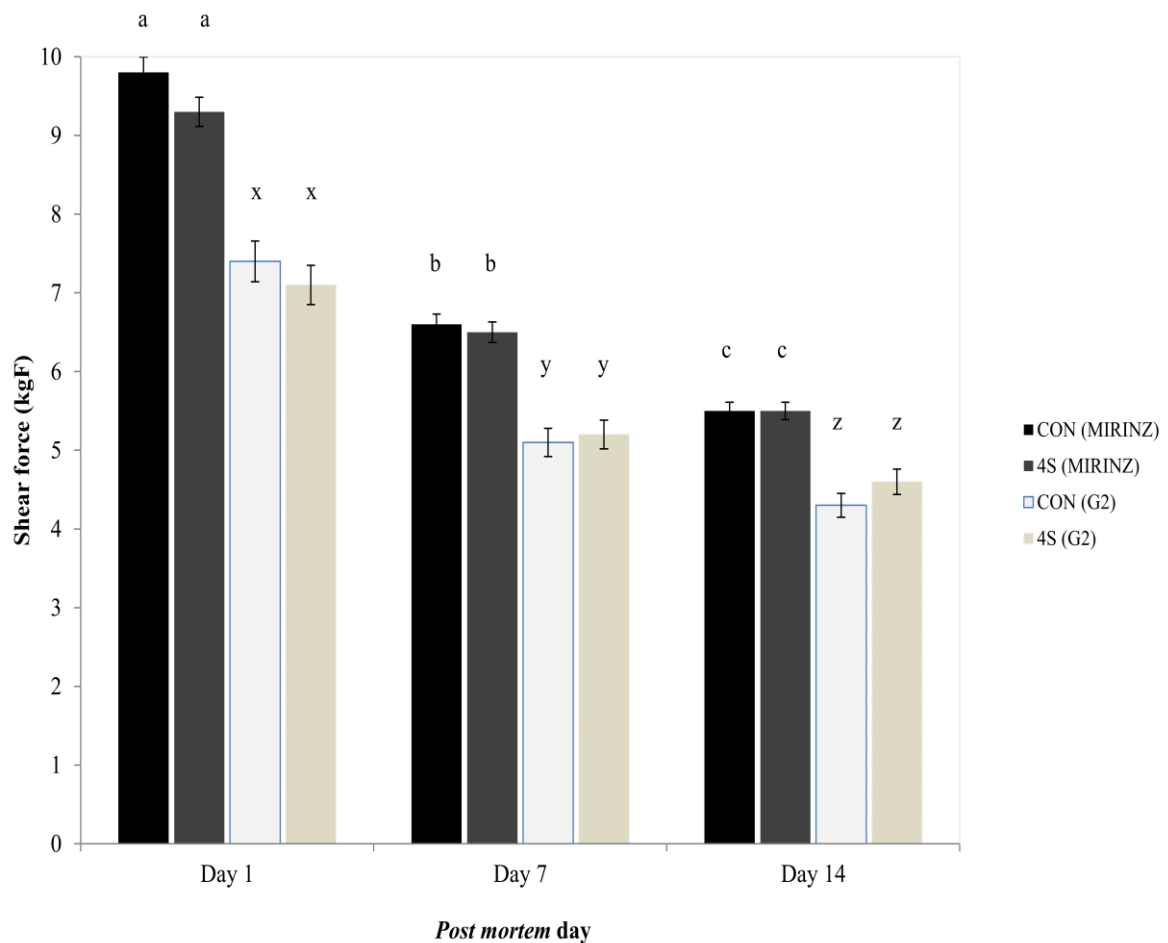
3.2. Shear force

4S treatment had no effect on mean shear force values ($P > 0.05$), while ageing resulted in a decrease ($P < 0.001$) in shear force values of the loins regardless of treatments (Figure 19). These results agreed with those of other studies with beef. Despite significant changes in dimensions and increases in sarcomere length of stretched hot-boned beef *M.*

semimembranosus and *M. longissimus lumborum* by 4S, no significant tenderness benefits were found (Toohey et al., 2010). Moreover, Taylor, Hopkins and van de Ven (2010) reported that there was no significant reduction in shear force values of beef *M. gluteus medius* and *M. semimembranosus* with 21% increase in length for both muscles arising from 4S treatment. Further, no significant sensory benefit from 4S treatment was reported for either beef primal (Taylor et al., 2010).

In contrast, a few experiments reported limited positive effects of 4S on meat tenderness. Taylor, Toohey and Hopkins (2011a) found the effect of 4S on tenderness development in hot-boned beef *M. gluteus medius* with 34% increase in length contributed to a

considerable decrease in shear force at 0 day *post mortem* but this effect was not evident at 8 days *post mortem*. Similarly, Toohey, van de Ven, Thompson, Geesink and Hopkins (2012) found that the 4S treatment of a whole sheep leg portion resulted in significant reductions of shear force values for *M. semimembranosus* and *M. biceps femoris* of up to 16% and 18.4%, respectively, after 0 day of ageing. Nevertheless, this 4S effect was nullified after 5 days of ageing. Interestingly, in their initial experiments, a promising significant tenderness improvement of sheep *M. semimembranosus* by 4S throughout the whole ageing period was found, and they concluded that 4S technology had a potential to replace ageing (Toohey et al., 2008b).



^{abc} Different letters indicate differences for the MIRINZ tenderometer ($P < 0.05$)

^{xyz} Different letters indicate differences for the G2 tenderometer ($P < 0.05$)

Figure 19. Mean shear force (kgF) of stretched (4S) and control (CON) samples for 1, 7 and 14 days *post mortem* measured with MIRINZ and G2 tenderometers.

Taken together, it can be proposed that a simple increase in muscle length by stretching *pre rigor* meat (whether it influences sarcomere length or not) would not always attribute to the meat tenderness improvement. In fact, Herring, Cassens, Suess, Brungardt and Briskey (1967) found that there was a curvilinear relationship between sarcomere length and tenderness, and thus they concluded that it would be more critical to prevent *post mortem* shortening than to ensure a maximum stretch. Further, they found fewer stretching effects on shear force and tenderness scores and a greater variability in shear force values in stretched beef samples from older carcasses (E-maturity; USDA) than ones from young carcasses (A-maturity; USDA) possibly due to different connective tissue contents and its flexibility (Herring et al., 1967). In that regards, Taylor and Hopkins (2011b) speculated that limited or no influence of 4S on meat tenderness could be due to a higher initial background toughness of meat from older animals. In general, beef obtained from physiologically mature carcasses was commonly observed to be tougher than the one from young carcasses due to greater connective tissue, in particular of higher amount of cross-linking (Shorthose & Harris, 1990). However, the current study examined beef muscles from 24 month-old steers, which were considered as young cattle, A- maturity, in the USDA grading system. Hence, a possible negation effect against *pre rigor* stretching due to greater connective contribution from of older animals is unlikely to be the case in the present study. Further, a possible interaction with electrical stimulation, which might reduce the effectiveness of 4S on tenderness is questionable, because no significant effect of 4S on shear force values were found regardless of applying electrical stimulation (Taylor et al., 2010) or not (Toohey et al., 2010).

Figure 20 combined the sarcomere length and shear force values of stretched muscles.

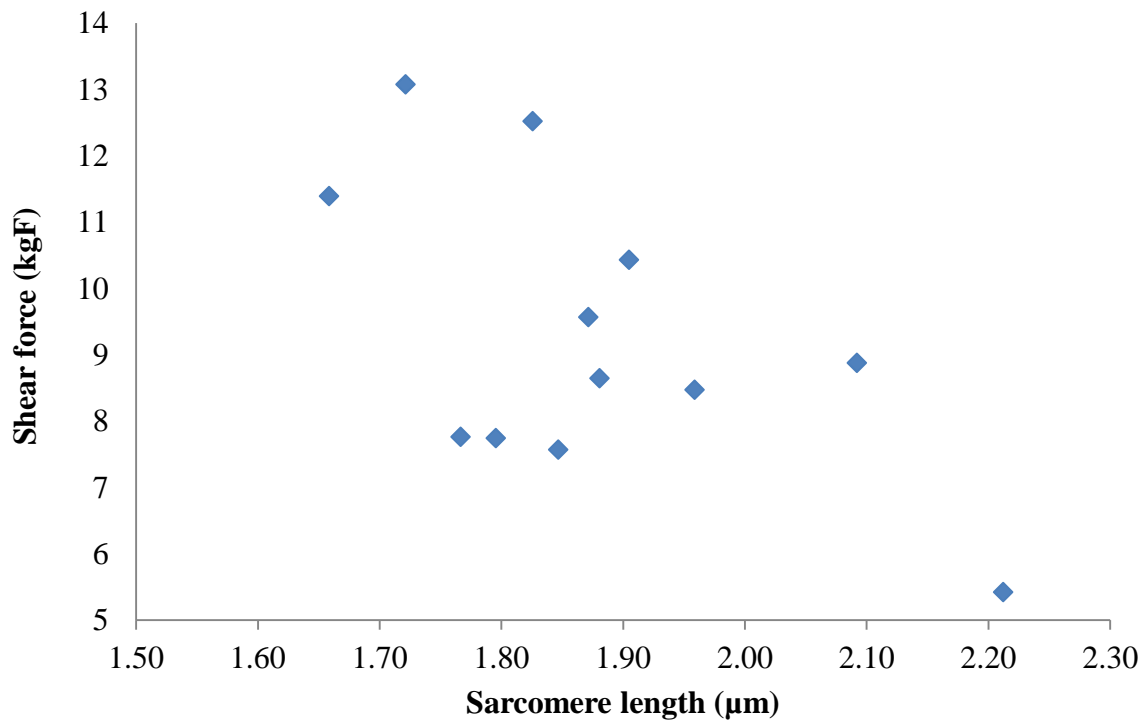


Figure 20. Relationship between shear force (kgF) and sarcomere length (μm). A linear equation fitted to these data had a correlation coefficient of $r = -0.608$ ($P < 0.05$).

Taylor, Toohey and Hopkins (2011a) found that increased sarcomere length resulted in decreased shear force values, regardless of treatment, with the strong negative correlation ($r = -0.61$). The result in Figure 20 was consistent with that finding.

Ageing times contributed to a significant reduction in shear force values regardless of the treatments, 9.8 kgF on 1 day to 5.5 kgF at 14 days *post mortem* based on the MIRINZ tenderometer and 7.3 kgF to 4.5 kgF based on the G2 tenderometer. Therefore, shear force values measured by G2 tenderometer could be multiplied by 1.25 to estimate shear force value from MIRINZ tenderometer just for guidance purpose because MIRINZ tenderometer has been extensively used as one of standard methods in tenderness-related studies in New Zealand (Bickerstaffe, Bekhit, Robertson, Roberts & Geesink, 2001). Hopkins, Toohey, Kerr and van de Ven (2011) compared the G2 tenderometer with a Lloyd texture analyser and found that the mean shear force values returned by a G2 tenderometer were about 1.3 times higher than values from the Lloyd texture analyser within less tender samples (shear force values < 50 Newtons or 5.01 kgF). They proposed

that shear force values measured by G2 tenderometer could be multiplied by 0.75-0.80 to estimate shear force values from Lloyd texture analyser within average tough samples (50 to 80 N) or (5.01 to 8.16 kgF). They suggested that different results from both machines could be due to shearing device in which Lloyd texture analyser possessing a sharper blade than G2 tenderometer having a much blunter shear blade.

3.3. Water-holding capacity

Purge and drip loss results indicated that there was no 4S effect on water-holding capacity ($P > 0.05$), which agrees with other results discussed above, while purge and drip loss increased with ageing time ($P < 0.05$; Table 1). Cooking loss data also revealed that there was no difference due to stretching treatment and ageing ($P > 0.05$). No significant effects of 4S on purge and cooking losses were also reported in beef (*M. gluteus medius* and *M. longissimus lumborum*) and sheep (*M. semimembranosus*) experiments (Taylor et al., 2011a). Similarly, Devine, Payne and Wells (2002b) also reported that cooking loss was not significantly different for a wrapped treatment, used to restrain muscle to prevent shortening, compared to a control treatment or ageing treatment in sheep (*M. longissimus*).

However, Simmons (2010) found purge loss was slightly higher in stretched muscle than non-stretched muscle. It was speculated that purge loss was more likely to increase due to sustained compression retained by packaging. Further, it was probably that more purge loss in stretched muscle was due to the stretched collagen resulting in reduction of volume of muscle (Simmons, 2010).

In addition, recent study by Taylor, Toohey and Hopkins (2011a) on hot-boned beef topside (*M. semimembranosus*) indicated that cooking loss was significantly decreased by stretching and increased due to ageing period with on average 40% increase in length. Additionally, Simmons (2010) found that cooking loss was positively affected by the stretching treatment. Cooking triggered control and stretched muscles to reduce in size since muscle collagen was denatured in both. Stretched samples had higher ability to retain their shape than controls after cooking. It was found that the stretched samples maintained a higher water content during cooking, and improved diameter revealed a potential to retain volume when heat stimulated contraction of collagen caused shortening on axis of muscle fibres (Simmons, 2010).

3.4. Proteolysis (desmin degradation)

Western blot assay found no stretching effect on extent of proteolysis. The present qualitative results indicated that there was no difference in desmin degradation between 4S and control loins throughout the whole ageing time (Figure 21). This observation clearly aligns with other physicochemical traits discussed above in that 4S did not affect sarcomere length and proteolysis, and subsequently did not attribute to influence water-holding capacity and shear force values. Taylor, Toohey and Hopkins (2011a) found similar results that 4S had no impact on proteolysis of beef *M. semimembranosus* and *M. longissimus lumborum* based on particle size analysis for protein degradation.

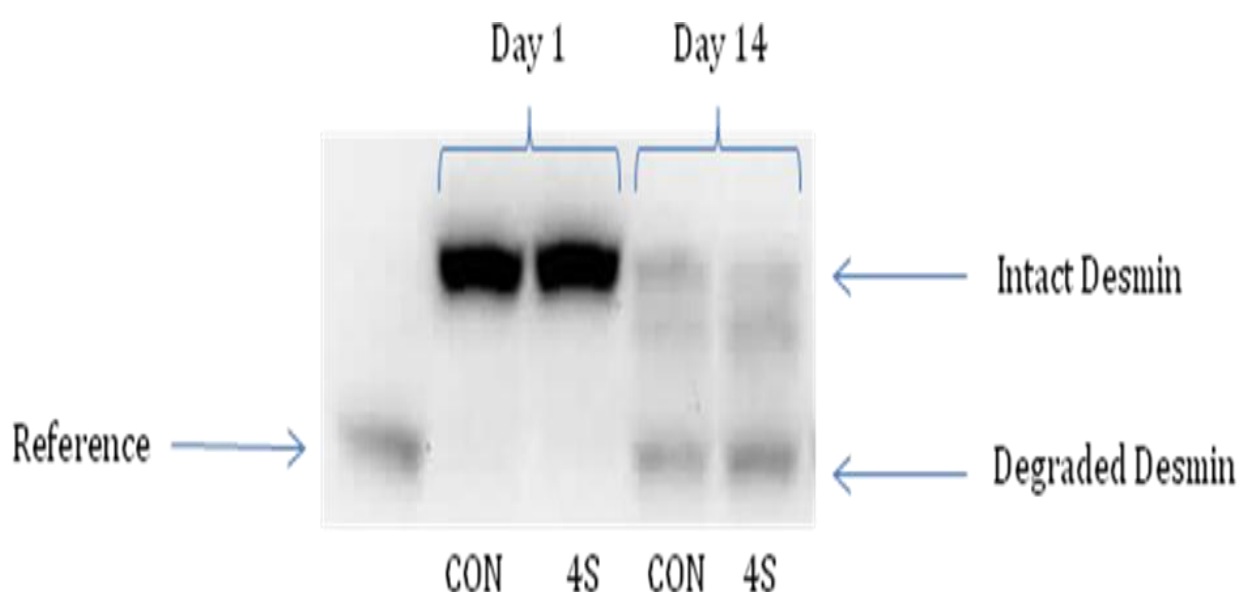


Figure 21. Representative Western blot desmin of whole muscle protein extracts from *M. longissimus* with control (CON) and stretched (4S) loin samples at different *post mortem* ageing periods (Days 1 and 14).

In contrast, Weaver, Bowker and Gerrard (2008) showed that sarcomere length (or degree of shortening) could be associated with the extent of proteolysis, thus influencing meat tenderness. They found that stretched beef samples (*M. semitendinosus*) held by clamps resulted in longer sarcomere length (2.57 μm), in which degradation products of myofibrillar proteins appeared at earlier time compared with samples induced to cold-shortening (1.43 μm). They explained this phenomenon by speculating a possible hindrance of potential proteolytic cleavage sites due to the greater proportion of actomyosin bonds in cold-shortened samples. It must be pointed out that the experiment was conducted to compare two extreme *pre rigor* conditions (clamp-stretched vs. cold-shortened muscles), which resulted in more than a 1 μm difference in sarcomere length (an 80% increase from

the cold-shortened length). Therefore, it is questionable if the suggested relationship between sarcomere length and proteolysis would be still valid when comparing stretched meat muscle with normal muscle, i.e. not cold-shortened. In fact, no close inter-relationship between sarcomere length and the extent of proteolysis has been reported by several studies (Jaime, Beltrán, Ceña, López-Lorenzo & Roncalés, 1992; Koohmaraie, Kennick, Anglemier, Elgasim & Jones, 1984; Wheeler & Koohmaraie, 1999), where the sarcomere difference was not greater than 1 μm between muscles tested. The current study did not even find a difference in sarcomere length between stretched and control samples, and thus it would not require further discussion about the relationship between sarcomere length and proteolysis.

Chapter 4

Conclusion

Beef loins were randomly allocated either to 4S technology or control. 4S technology contributed to different percentages increase in stretched *M. longissimus dorsi* muscles, on average 33% at 0 day of ageing. 4S technology generated the definite shapes of meat loins. Controls were wrapped by cling polyethylene film to prevent dehydration of meat cuts.

However, this change did not affect the biophysical properties of *post mortem* beef muscles as shown by no differences in sarcomere length, water-holding capacity (purge, drip and cooking losses), shear force values and proteolysis between 4S and control samples.

However, shear force values were significantly reduced in response to ageing times regardless of the stretching treatment. It remains unclear for the possible explanation of no impact of 4S on either sarcomere length or tenderness of beef loins at the current muscle length change from this study, whilst positive results of 4S on meat tenderness from sheep meat studies were observed even under less change in muscle length achieved by 4S.

Therefore, further studies regarding the effects of different extents of stretching, muscle types, age/breed types of cattle, *pre rigor* pH values, *pre rigor* and ageing temperatures and electrical stimulation would be warranted.

References

- Aberle, E. D., Forrest, J. C., Gerrard, D. E., & Mills, E. W. (2001). *Meat Science*. Dubuque, IA: Kendall/Hunt Publishing Company.
- Anonymous (n.d.). Sarcoplasmic reticulum and transverse tubules
- Arcus, A. C. (1959). Proteolytic enzyme of *Actinidia chinensis*. *biochemica et Biophysica Acta*, 33, 242-244.
- Baechle, T. R., & Earle, R. W. (2008). *Essentials of strength training and conditioning*. Champaign, Illinois: Human Kinetics.
- Barnier, V. M. H., Wiklund, E., Vandijk, A., Smulders, F. J. M., & Malmfors, G. (1999). Proteolytic enzyme and inhibitor levels in reindeer (*Rangifer tarandus tarandus*) vs. bovine longissimus muscle, as they relate to ageing rate and response. *Rangifer*, 19, 13-18.
- Beaty, S. L., Apple, J. K., Rakes, L. K., & Kreider, D. L. (1999). Early postmortem skeletal alterations effect on sarcomere length, myofibrillar fragmentation, and muscle tenderness of beef from light-weight, Brangus heifers. *Journal of Muscle Foods*, 10, 67-78.
- Beef + Lamb New Zealand (2011). Sheep and beef new season outlook 2011-2012.
- Belitz, H.-D., Grosch, W., & Schieberle, P. (2009). *Food Chemistry*. Leipzig: Springer-Verlag Berlin Heidelberg.
- Bendall, J. R. (1967). The elastin content of various muscles of beef animals. *Journal of Science of Food and Agriculture*, 18, 553-558.
- Bendall, J. R. (1978). Variability in rates of pH fall and of lactate production in the muscles on cooling beef carcasses. *Meat Science*, 2, 91-104.
- Bickerstaffe, R., Bekhit, A. E. D., Robertson, L. J., Roberts, N., & Geesink, G. H. (2001). Impact of introducing specifications on the tenderness of retail meat. *Meat Science*, 59, 303-315.
- Bouton, P. E., Fisher, A., Harris, P. V., & Baxter, R. I. (1973a). A comparison of the effects of some post-slaughter treatments on the tenderness of beef. *Journal of Food Technology*, 8, 39-49.
- Bouton, P. E., Harris, P. V., Shorthose, W. R., & Baxter, R. I. (1973b). A comparison of the effects of ageing, conditioning and skeletal restraint on the tenderness of mutton. *Journal of Food Science*, 38, 932-937.
- Bruce, H. L., & Ball, R. O. (1990). Postmortem interactions of muscle temperature, ph and extension of beef quality. *Journal of Animal Science*, 68(12), 4167-4175.
- Carse, W. A. (1973). Meat quality and the acceleration of postmortem glycolysis by electrical stimulation. *Journal of Food Technology*, 8, 163-166.
- Chrystall, B., & Devine, C. E. (1975). Electrical stimulation in New Zealand. In: *Meat Science and Technology Symposium* (p. 115). Chicago: National Livestock and Meat Board.
- Chrystall, B. B., & Devine, C. E. (1983). Electrical stimulation of deer carcasses. *New Zealand Journal of Agricultural Research*, 26, 89-92.
- Chrystall, B. B., & Hagyard, C. J. (1976). Electrical stimulation and lamb tenderness. *New Zealand Journal of Agricultural Research*, 19, 7-11.
- Claus, J. R. (2002). The tendercut process.
- Claus, J. R., Wang, H., & Marriott, N. G. (1997). Prerigor carcass muscle stretching effects on tenderness of grain-fed beef under commercial conditions. *Journal of Food Science*, 62, 1231-1234.

- Daly, C. C., Simmons, N. J., & Devine, C. E. (1995). An interaction of pre-slaughter stress and electrical stimulation on the tenderness of lamb. *41st International Congress of Meat Science and Technology*. San Antonio, Texas.
- Davey, C. L., & Gilbert, K. V. (1976). The temperature coefficient of beef ageing. *Journal of Science of Food and Agriculture*, 27, 244-250.
- Davey, C. L., Gilbert, K. V., & Carse, W. A. (1976). Carcass electrical stimulation to prevent cold shortening toughness in beef. *New Zealand Journal of Agricultural Research*, 19, 13-18.
- Davies, A. S. (2004). Muscle structure and contraction. In: W. J. Jensen, C. E. Devine, & M. E. Dikeman, *Encyclopedia of Meat Science* (pp. 882-901). Oxford: Elsevier Science.
- Deng, Y., Rosenvold, K., Karlsson, A. H., Horn, P., Hedegaard, J., Steffensen, C. L., & al., e. (2002). Relationship between thermal denaturation of porcine muscle proteins and water-holding capacity. *Journal of Food Science*, 67(5), 1642-1647.
- Devine, C. E., & Graafhuis, A. E. (1995). The basal toughness of unaged lamb. *Meat Science*, 39, 285-291.
- Devine, C. E., Lowe, T. E., Wells, R. W., Edwards, N. J., Edwards, J. E. H., Starbuck, T. J., & Speck, P. A. (2006). Pre-slaughter stress arising from on-farm handling and its interactions with electrical stimulation on tenderness of lambs. *Meat Science*, 73, 304-312.
- Devine, C. E., Payne, S. R., Peachey, B. M., Lowe, T. E., Ingram, J. R., & Cook, C. J. (2002a). High and low rigor temperature effects on sheep meat tenderness and ageing. *Meat Science*, 60(2), 141-146.
- Devine, C. E., Payne, S. R., & Wells, R. W. (2002b). Effect of muscle restraint on sheep meat tenderness with *rigor mortis* at 18°C. *Meat Science*, 60(2), 155-159.
- Devine, C. E., Wahlgren, N. M., & Tornberg, E. (1999). Effect of rigor temperature on muscle shortening and tenderisation of restrained and unrestrained beef *m.longissimus thoracicus et lumborum*. *Meat Science*, 51, 61-72.
- Dikeman, M. E. (1987). Fat reduction in animals and the effects on palatability and consumer acceptance of meat products. *Reciprocal Meat Conference*, 40, 93-103.
- Dransfield, E. (1994). Optimisation of Tenderisation, Ageing and Tenderness. *Meat Science*, 36, 105-121.
- Dransfield, E., Ledwith, M. J., & Taylor, A. A. (1991). Effect of electrical stimulation, hip suspension and ageing on quality of chilled pig meat. *Meat Science*, 29, 129-139.
- Drew, K. R., Crosbie, S. F., Forss, D. A., Manley, T. R., & Pearse, A. J. (1988). Electrical stimulation and ageing of carcasses from red, fallow and New Zealand wapiti-type male deer. *Journal of Science of Food and Agriculture*, 43, 245-259.
- Dulhunty, A. F., Haarmann, C. S., Green, D., Laver, D. R., & Casaratto, M. G. (2002). Interaction between dihydropyridine receptors and ryanodine receptors in striated muscle. *Progress in Biophysics and Molecular Biology*, 79, 45-75.
- Dutson, T. R., Smith, G. C., Savell, J. W., & Carpenter, Z. L. (1980). Possible mechanism by which electrical stimulation improve meat tenderness. In: *the 26th European Meat Researchers and Workers Conference* (pp. 84-87). Colorado Springs, USA.
- Etherington, D. J. (1987). Collagen and meat quality: effects of conditioning and growth rate. In: A. M. Pearson, & T. R. Dutson, *Advance in Meat Research*, vol. 4 (pp. 351-560). New York: van Nostrand Reinhold.
- Farouk, M. M., Beggan, M., Hurst, S., Staurt, A., Debbie, P. M., & Bekhit, A. E. D. (2007). Meat quality attributes of chilled venison and beef. *Journal of Food Quality*, 30, 1023-1039.

- Farouk, M. M., Wiklund, E., & Rosenvold, K. (2009). Carcass interventions and meat tenderness. In: J. Kerry, & D. Ledward, *Improving the sensory and nutritional quality of fresh meat*. Cambridge, England: Woodhead Publishing Limited.
- Feiner, G. (2006). *Meat products handbook*. New York: Woodhead Publishing Limited and CRC Press LLC.
- Fiems, L. O., Batjoens, P., Uytterhaegen, L., Moermans, R. J., Van Hoof, J., & Boucque, C. V. (1993). The effect of cimeterol on myofibrils and metabolic aspects in different strains of Belgian white-blue bulls., *39th International Congress of Meat Science and Technology*. Calgary, Canada.
- Fiems, L. O., Buts, B., Boucque, C. V., Demeyer, D. I., & Cottyn, B. G. (1990). Effect of a beta-agonist on meat quality and myofibrillar protein degradation in bulls. *Meat Science*, 27, 29-39.
- Foegeding, E. A., Lanier, T. C., & Hultin, H. O. (1996). Characteristics of Edible Muscle Tissues. In: O. R. Fennema, *Food Chemistry*. New York: Marcel Dekker, Inc.
- Geesink, G. (1993). Post mortem muscle proteolysis and beef tenderness with special reference to the action of the calpain/calpastatin system. vol. PhD Thesis: Utrecht University.
- Geesink, G. H., Bekhit, A. E. D., & Bickerstaffe, R. (2000). Rigor temperature and meat quality characteristics of lamb longissimus muscle. *Journal of Animal Science*, 78(11), 2842-2848.
- Geesink, G. H., VanLaack, R. L. J. M., Barnier, V. M. H., & Smulders, F. J. M. (1994). Does electrical stimulation affect the speed of ageing or ageing response? *Science des Aliments*, 14, 409-422.
- Goll, D. E., Thompson, V. F., Li, H., Wei, W. E. I., & Cong, J. (2003). The Calpain system. *Physiological Reviews*, 83(3), 731-801.
- Harris, P. V. (1974). Meat chilling. *Commonwealth Scientific Industry Research Organization Food Research Quartely*, 35, 49-56.
- Herring, H. K., Cassens, R. G., & Briskey, E. J. (1965). Further studies on bovine muscle tenderness as influenced by carcass position, sarcomere length, and fiber diameter. *Journal of Animal Science*, 30(6), 1049-1054.
- Herring, H. K., Cassens, R. G., Suess, C. G., Brungardt, V. H., & Briskey, E. J. (1967). Tenderness and associated characteristics of stretched and contracted bovine muscle. *Journal of Food Technology*, 32, 317-323.
- Herring, J. L., & Rogers, R. W. (2003). Evaluation of cooking methods on various beef steaks. *Journal of Muscle Foods*, 14, 163-171.
- Highfill, C. M., Esquivel-Font, O., Dikeman, M. E., & Kropf, D. H. (2012). Tenderness profiles of ten muscle from F1 *Bos indicus* x *Bos taurus* and *Bos taurus* cattle cooked as steaks and roasts. *Meat Science*, 90(4), 881-886.
- Hildrum, K. I., Andersen, T., Nilsen, B. N., & Wahlgren, M. (2000). Improving beef tenderness by restricting rigor mortis contraction. *Proceeding of the 46th International Congress of Meat Science and Technology* (pp. 444-445). Buenos Aires.
- Hildrum, K. I., Nilsen, B. N., & Wahlgren, M. (2002). Improving the tenderness of hot boned beef muscles. *Fleischwirtsch International*(1), 42-48.
- Ho, C. Y., Stromer, M. H., & Robson, R. M. (1996). Effect of electrical stimulation on postmortem titin, nebulin, desmin, and troponin T degradation and ultrastructural changes in bovine longissimus muscle. *Journal of Animal Science*, 74, 1563-1575.
- Honikel, K. O. (1998). Reference Methods for the assessment of Physical Characteristic of Meat. *Meat Science*, 49(4), 447-457.
- Honikel, K. O. (2004). Conversion of muscle to meat. In: W. K. Jensen, C. E. Devine, & M. Dikeman, *Encyclopedia of meat science*: Elsevier Academic Press.

- Hopkins, D. L. (2004). Tenderizing mechanisms - mechanical. In: J. Werner Klith, *Encyclopedia of Meat Sciences* (pp. 1355-1363). Oxford: Elsevier.
- Hopkins, D. L., Garlicks, P. R., & Thompson, J. M. (2000). The effect on the sarcomere structure of super tenderstretching. *Journal of Animal Science*, C, 233.
- Hopkins, D. L., & Thompson, J. M. (2001). The relationship between tenderness, proteolysis, muscle contraction and dissociation of actomyosin. *Meat Science*, 57(1), 1-12.
- Hopkins, D. L., Toohey, E. S., Kerr, M. J., & Ven, R. v. d. (2011). Comparison of two instruments (G2 Tenderometer and a Lloyd Texture analyser) for measuring the shear force of cooked meat. *Animal Production Science*, 51, 71-76.
- Hopkinson, S. F., Ringkob, T. P., & Bailey, C. M. (1985). Cutability and the effect of electrical stimulation and ageing on tenderness of beef from young intact males and castrates. *Journal of Animal Science*, 60, 675-681.
- Hostetler, R. L., Link, B. A., Landmann, W. A., & Fitzhugh, H. A. (1972). Effect of carcass suspension on sarcomere length and shear force of some major *bovine* muscle. *Journal of Food Science*, 37(1), 132-135.
- Huff-Lonergan, E., Mitsuhashi, T., Beekman, D. D., Parrish, F. C., Jr., Olson, D. G., & Robson, R. M. (1996). Proteolysis of specific muscle structural proteins by mu-calpains at low pH and temperature is similar to degradation in *post mortem* bovine muscle. *Journal of Animal Science*, 74(5), 993-1008.
- Huxley, A. F., & Hanson, J. (1954). Changes in the cross striations of muscle during contraction and stretch and their structural interpretation. *Nature*, 173, 973-976.
- Hwang, I. H., Devine, C. E., & Hopkins, D. L. (2003). The biochemical and physical effects of electrical stimulation on beef and sheep meat tenderness. *Meat Science*, 65(2), 677-691.
- Hwang, I. H., & Thompson, J. M. (2001). The interaction between pH and temperature decline early postmortem on the calpain system and objective tenderness in electrically stimulated beef *longissimus dorsi* muscle. *Meat Science*, 58(2), 167-174.
- Immonen, K., & Poulanne, E. (2000). Variation of residual glycogen-glucose concentration at ultimate pH values below 5.75. *Meat Science*, 58(1), 17-23.
- Jaime, I., Beltrán, J. A., Ceña, P., López-Lorenzo, P., & Roncalés, P. (1992). Tenderisation of lamb meat: Effect of rapid postmortem temperature drop on muscle conditioning and aging. *Meat Science*, 32(4), 357-366.
- Jeremiah, L. E., Gibson, L. L., & Cunningham, B. (1999). The influence of mechanical tenderization on the palatability of certain bovine muscles. *Food Research International*, 32, 585-591.
- Joseph, R. L., & Connolly, J. (1976). The effect of suspension method, chilling rate and post mortem ageing period on beef quality. *Journal of Food Technology*, 12, 231-247.
- Kim, Y. H., Lonergan, S. M., & Huff-Lonergan, E. (2010). Protein denaturing conditions in beef deep semimembranosus muscle results in limited μ -calpain activation and protein degradation. *Meat Science*, 83(3), 883-887.
- Koohmaraie, M. (1988). The role of endogenous proteases in meat tenderness. In: *the 41st annual Reciprocal Meat Conference* (pp. 89-100). Laramie, WY.
- Koohmaraie, M. (1996). Biochemical Factors Regulating the Toughening and Tenderization Processes of Meat. *Meat Science*, 43, S193-S201.
- Koohmaraie, M., Doumit, M. E., & Wheeler, T. L. (1996). Meat toughening does not occur when rigor shortening is prevented. *Journal of Animal Science*, 74, 2935-2942.
- Koohmaraie, M., Kennick, W. H., Anglemier, A. F., Elgasim, E. A., & Jones, T. K. (1984). Effect of postmortem storage on cold-shortened bovine muscle: Analysis by SDS-polyacrylamide gel electrophoresis. *Journal of Food Science*, 49, 290-291.

- Koohmaraie, M., Shackelford, S. D., & Wheeler, T. L. (1998). The biological basis of beef tenderness and potential approaches for its control and prediction. Sao Palo, Brazil: Presentation at Fundeppec Symposium.
- Kretchmar, D. H., Hathaway, M. R., Epley, R. J., & Dayton, W. R. (1990). Alterations in post mortem degradation of myofibrillar proteins in muscle of lambs fed a beta-adrenergic agonist. *Journal of Animal Science*, 68, 1760-1772.
- Lawrie, R. A. (1998). *Meat Science*. Cambridge: Woodhead Publishing Limited.
- Lawrie, R. A., & Ledward, D. A. (2006). *Lawrie's meat science*. Cambridge: Woodhead Publishing Limited.
- Lee, Y. B., & Ashmore, C. R. (1985). Effect of early postmortem temperature on beef tenderness. *Journal of Animal Science*, 60(6), 1588-1596.
- Listrat, A., Lethias, C., Hocquette, J. F., Renand, G., Mennissier, G., & Geay, Y. (2000). Age-related changes and location of types I, III, XII and XIV collagen during development of skeletal muscles from genetically different animals. *Histochemical Journal*, 32, 349-356.
- Locker, R. H., & Hagyard, C. J. (1963). A cold shortening effect in beef muscle. *Journal of the Science of Food and Agriculture*, 14(11), 787-793.
- Lonergan, S. M., Huff-Lonergan, E., Rowe, L. J., Kuhlbers, D. L., & Jungst, S. B. (2001). Selection of lean growth efficiency in Duroc pigs influence pork quality. *Journal of Animal Science*, 79(8), 2075-2085.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the folin phenol reagent. *The Journal of Biological Chemistry*, 193(1), 265-275.
- Ludwig, C. J., Claus, J. R., Marriott, N. G., Johnson, J., & Wang, H. (1997). Skeletal alteration to improve beef *longissimus* muscle tenderness. *Journal of Animal Science*, 75, 2404-2410.
- Lusk, J. L., Fox, J. A., Schroeder, T. C., Mintert, J., & Koohmaraie, M. (2001). In-store valuation of steak tenderness. *American Journal of Agricultural Economics*, 83, 539-550.
- Macfarlane, P. G., & Marer, J. M. (1966). An apparatus for determining the tenderness of meat. *Food Technology*, 839, 134-135.
- Maddock, R. (2008). Mechanical Tenderization of Beef. (pp. 1-4): Cattlemen's Beef Board and National Cattlemen's Beef Association.
- Marsh, B. B., & Carse, W. A. (1974). Meat tenderness and the sliding-filament hypothesis. *Journal of Food Technology*, 9, 129-139.
- Marsh, B. B., & Leet, N. G. (1966). Resistance to shearing of heat-denatured muscle in relation to shortening. *Nature*, 211, 635-636.
- McCormick, R. j. (1999). Extracellular modifications to muscle collagen: Implications for meat quality. *Poultry Science*, 78, 785-791.
- Meat Industry Association (2009). *MEAT IN FOCUS*.
- Meat Industry Association (2011). 2011 Annual Report.
- Meixner, H.-W., & Karnitzschky, I. (2001). Warmfleisch richtig verpackt. *Fleischwirtschaft*, 81, 30-31.
- Mickelson, J. R. (1983). Calcium transport by bovine skeletal-muscle mitochondria and its relationship to post-mortem muscle. *Meat Science*, 9(3), 205-229.
- Miller, R. (2003). Assessing consumer preferences and attitudes toward meat and meat products. *Brazilian Journal of Food Technology*, 6, 67-80.
- Miller, R. K. (2004). Chemical and physical characteristics of meat | Palatability. In: J. Werner Klinth, *Encyclopedia of Meat Sciences* (pp. 256-266). Oxford: Elsevier.

- Moller, A. J., & Vestergaard, T. (1986). Effect of altered carcass suspension during rigor mortis on tenderness of pork loin. *Meat Science*, 18, 77-87.
- Ngapo, T. M., & Dransfield, E. (2006). British consumers preferred fatness levels in beef: surveys from 1955, 1982 ad 2002. *Food Quality and Preference*, 17(5), 412-417.
- Nishimaya, I. (2001). pH-dependent proteolytic effects of actinidin on myofibrillar proteins. *Journal of Home Economic of Japan*, 52(11), 1083-1089.
- Ouali, A. (1992). Proteolytic and physicochemical mechanisms involved in meat texture development. *Biochimie*, 74, 251-265.
- Parrish, F. C., Vandell, C. J., & Culler, R. D. (1979). Effect of maturity and marbling on the myofibril fragmentation index of bovine longissimus muscle. *Journal of Food Science*, 48, 1668-1671.
- Pearson, A. M., Carse, W. A., Davey, C. L., Locker, R. H., & Hagyard, C. J. (1973). Influence of epinephrine and calcium upon glycolysis, tenderness and shortening of sheep muscle *Journal of Food Science*, 38, 1124-1127.
- Pearson, A. M., & Young, R. B. (1989). *Muscle and Meat Biochemistry*. SanDiego, CA: Academic Press, Inc.
- Pedersen, M. E., Kolset, S. O., Sorensen, T., & Eggen, K. H. (1999). Sulfated glycosaminoglycans and collagen in two bovine muscle (*M. Semitendonsus* and *M. Psoas Major*) differing in texture *Journal of Agricultural and Food Chemistry*, 47, 1445-1452.
- Pethick, D. W., Harper, G. S., & Oddy, V. H. (2004). Growth, development and nutritional manipulation of marbling in cattleL a review. *Australian Journal of Experimental Agriculture*, 44(7), 705-715.
- Pitt, A., & Daly, C. C. (2010). Meat Stretching device and method. vol. US20100166931.
- Quarrier, E., Carpenter, Z. L., & Smith, G. C. (1972). A physical method to increase tenderness in lamb carcass. *Journal of Food Science*, 37, 130-131.
- Rawn, D. J. (1989). *Biochemistry*. NC, USA: Neil Patterson Publishers.
- Robson, R. M. (1991). Biochemical and structural properties of titin, nebulin and intermediate filaments of muscle. *Proceedings Reciprocal Meat Conference*, 44, 7.
- Rødbotton, R., Lea, P., & Hildrum, K. I. (2001). Relative influence of low-voltage electrical stimulation, chilling rate and ageing time on tenderness of *M.longissimus dorsi* of Norwegian cattle. *Acta Agriculturae Scandinavica, A*, 51, 184-191.
- Rosenvold, K., & Andersen, H. J. (2003). Factors of significance for pork quality- A review. *Meat Science*, 64, 219-237.
- Rosenvold, K., North, M., Devine, C. E., Micklander, E., Hansen, P. W., Dobbie, P. M., & Wells, R. (2008). The protective effect of electrical stimulation and wrapping on beef tenderness at high pre-rigor temperatures. *Meat Science*, 79, 299-306.
- Rossi, A. E., & Dirksen, R. T. (2006). Sarcoplasmic reticulum: The dynamic calcium governor of muscle. *Muscle Nerve*, 33, 715-731.
- SAS (2007). SAS Institute Incorporated. Cary, NC, USA.
- Savell, J. W., McKeith, F. K., & Smith, G. C. (1981). Reducing postmortem aging time of beef with electrical stimulation. *Journal of Food Science*, 46, 1777-1781.
- Savell, J. W., Mueller, S. L., & Baird, B. E. (2005). The chilling of carcasses. *Meat Science*, 70, 449-459.
- Sensky, P. L., Parr, T., Bardsley, R. G., & Buttery, P. J. (1996). The relationship between plasma epinephrine concentration and the activity of the calpain enzyme system in porcine *longissimus* muscle. *Journal of Animal Science*, 74, 380-387.
- Sentandreu, M. A., Coulis, G., & Ouali, A. (2002). Role of muscle endopeptidases and their inhibitors in meat tenderness. *Trends in Food Science and Technology*, 13, 398-419.

- Shorthose, W. R., & Harris, P. V. (1990). Effect of Animal Age on the Tenderness of Selected Beef Muscles. *Journal of Food Science*, 55(1), 1-9.
- Simmons, N. (2010). Smartstretch. New South Wales: Meat & Livestock Australia.
- Simmons, N. J., Cairney, J. M., Auld, M. M., Nagle, T. A., & Mudford, C. R. (1999). Effect of prerigor stretching of beef *M. longissimus thoracis* muscles on structural changes and key meat quality attributes. In: *the 45th International Congress of Meat Science and Technology* Yokohama, Japan.
- Simmons, N. J., Daly, C. C., Mudford, C. R., Richards, I., Jarvis, G., & Pleiter, H. (2006). Intergrated technologies to enhance meat quality - an australasian perspective. *Meat Science*, 74(1), 172-179.
- Simmons, N. J., Young, O. A., Dobbie, P. M., Singh, K., Thompson, B. C., & Speck, P. A. (1997). Post-mortem calpain-system kinetics in lamb: effects of clenbuterol and preslaughter exercise. *Meat Science*, 47, 135-146.
- Siver, F. H., Kato, Y. P., Ohno, M., & Wasserman, A. J. (1992). Analysis of mammalian connective tissue: Relationship between hierarchiral structures and mechanical properties. *Journal of Long Term Effects of Medical Implants*, 2, 165-198.
- Smet, S. D. (2004). Double-muscled animals. In: W. J. Jensen, C. E. Devine, & M. E. Dikeman, *Encyclopedia of Meat Science*. Oxford: Elsevier Science.
- Smulders, F. J. M., Barnier, V. M. H., Geesink, G. H., & van Laack, R. (1995). The muscle biological background of meat tenderness. In: K. Lundstrom, I. Hannsson, & E. Wiklund, *Composition of Meat in Relationship to Processing, Nutritional and Sensory Quality. From Farm to Fork*. Utrecht: ECCEAMST.
- Smulders, F. J. M., Marsh, B. B., Swartz, D. R., Russell, R. L., & Hoenecke, M. E. (1990). Beef Tenderness and Sarcomere Length. *Meat Science*, 28, 349-363.
- Smulders, F. J. M., Toldra, F., Flores, J., & Prieto, M. (1992). *New Technologies for Meat and Meat Products*. Utrecht, The Netherlands: Audet Tijdschriften.
- Sorheim, O., & Hildrum, K. I. (2002). Muscle stretching techniques for improving meat tenderness. *Trend in Food Science & Technology*, 13, 127-135.
- Sørheim, O., Idland, J., Halvorsen, E. C., Frøystein, T., Lea, P., & Hildrum, K. I. (2001). Influence of beef carcass stretching and chilling rate on tenderness of *m. longissimus dorsi*. *Meat Science*, 57, 79-85.
- Strasburg, G., Xiong, Y. L., & Chiang, W. (2007). Postmortem Physiology of Edible Muscle Tissues. In: S. Damodaran, K. L. Parkin, & O. R. Fennema, *Fennema's Food Chemistry*. Florida: CRC Press.
- Taylor, A. A., Perry, A. M., & Warkup, C. C. (1995). Improving pork quality by electrical stimulation or pelvic suspension of carcasses. *Meat Science*, 39, 327-337.
- Taylor, J., Toohey, E., & Hopkins, D. L. (2011a). Accelerated processing of sheep/beef meat SmartStretchTM/SmartShapeTM. New South Wales: Meat & Livestock Australian Limited.
- Taylor, J. M., & Hopkins, D. L. (2011b). Patents for stretching and shaping meats. *Recent patents on Food, Nutrition & Agriculture*, 3, 91-101.
- Taylor, J. M., Hopkins, D. L., & van de Ven, R. (2010). The effect of a meat stretching device on the tenderness of hot-boned beef topsides and rostbiffs. In: *56th International Congress of Meat Science and Technology*. Jeju, Korea.
- Taylor, R. G. (2003). Meat tenderness: Theory and Practice. *Brazilian Journal of Food Technology*, 6, 56-66.
- Taylor, R. G. (2004). Connective tissue structure, function and influence on meat quality. In: W. J. Jensen, C. E. Devine, & M. E. Dikeman, *Encyclopedia of Meat Science* (pp. 306-313). Oxford: Elsevier Science.

- Taylor, J., Toohey, E. S., Ven, R. v. d., & Hopkins, D. L. (2012). SmartStretch™ technology. IV. The impact of the meat quality of hot-boned beef rosbiff (*m. gluteus medius*). *Meat Science*, 91(4), 527-532.
- Toohey, E., Hopkins, D., & Lamb, T. A. (2008a). The Impact of wrapping and ageing hot boned sheep meat on eating quality. In: *the 54th International Congress of Meat Science and Technology* (p. 7B.19). Capetown, South Africa.
- Toohey, E. S., Hopkins, D. L., Lamb, T. A., Nielsen, S. G., & Gutzke, D. (2008b). Accelerated tenderness of sheep topsides using a meat stretching device. In: *the 54th International Congress of Meat Science and Technology*. Capetown, South Africa.
- Toohey, E. S., Kerr, M. J., van de Ven, R., & Hopkins, D. L. (2010). The effect of smartstretch technology on the tenderness of beef topsides and cube rolls. In: *the 56th International Congress of Meat Science and Technology*. Jeju, Korea.
- Toohey, E. S., Ven, R. v. d., Thompson, J. M., Geesink, G. H., & Hopkins, D. L. (2012). SmartStretch™ Technology: V. The impact of SmartStretch™ technology on beef topsides (*m. semimembranosus*) meat quality traits under commercial processing conditions *Meat Science*, 92(1), 24-29.
- Torrescano, G., Sánchez-Escalante, A., Giménez, B., Roncalés, P., & Beltrán, J. A. (2003). Shear values of raw samples of 14 bovine muscles and their relation to muscle collagen characteristics. *Meat Science*, 64(1), 85-91.
- Tortora, G. J., & Derrickson, B. (2006). *Principles of Anatomy and Physiology*. Hoboken, NJ: John Wiley and Sons, Inc.
- USDA (1997). Official United States standards for grades of carcass beef. Washington, DC: Agricultural Marketing Service, United States Department of Agriculture.
- Uytterhaegen, L., Claeys, E., Demeyer, D., Lippens, M., Fiems, L. O., Boucque, C. Y., Van de Voorde, R., & Bastiaens, A. (1994). Effects of double-muscling on carcass quality, beef tenderness and myofibrillar protein degradation in Belgian blue white bulls.
- Vidalenc, P., Cottin, P., Merdaci, N., & Ducastaing, A. (1983). Stability of two Ca²⁺ dependent neutral proteinases and their specific inhibitors during post-mortem storage of rabbit skeletal muscle. *Journal of the Science of Food and Agriculture*, 34, 1241-1250.
- Wahlgren, N. M., & Hildrum, K. I. (2001). Improving the tenderness of hot-boned strip loins using a novel packaging method. *Proceeding of the 47th International Congress of Meat Science and Technology* (pp. 116-117). Krakow.
- Wang, H., Claus, J. R., & Marriott, N. G. (1994). Selected skeletal alterations to improve tenderness of beef round muscle. *Journal of Muscle Foods*, 5, 137-147.
- Wang, H., Claus, J. R., & Marriott, N. G. (1995). A research note - tenderness of prerigor stretched porcine *longissimus* muscle. *Journal of Muscle Foods*, 6, 75-82.
- Warriss, P. D. (2000). *MEAT SCIENCE An Introductory Text*. New York: CABI Publishing.
- Watanabe, A., Daly, C. C., & Devine, C. E. (1996). The effects of the ultimate pH of meat on tenderness change during aging. *Meat Science*, 42, 67-78.
- Weaver, A. D., Bowker, B. C., & Gerrard, D. E. (2008). Sarcomere length influences postmortem proteolysis of excised bovine semitendinosus muscle. *Journal of Animal Science*, 86, 1925-1932.
- Webb, E. C., & O'Neill, H. A. (2008). The animal fat paradox and meat quality. *Meat Science*, 80(1), 28-36.
- Wheeler, T. L., & Koohmaraie, M. (1992). Effects of the beta-adrenergic agonist L_{644,969} on muscle protein turnover, endogenous proteinase activities and meat tenderness in steers. *Journal of Animal Science*, 70, 3035-3043.

- Wheeler, T. L., & Koohmaraie, M. (1994). Prerigor and postrigor changes in tenderness of ovine longissimus muscle. *Journal of Animal Science*, 72, 1232-1238.
- Wheeler, T. L., & Koohmaraie, M. (1999). The extent of proteolysis is independent of sarcomere length in lamb and psoas major. *Journal of Animal Science*, 77, 2444-2451.
- Wheeler, T. L., Savell, J. W., Cross, H. R., Lunt, D. K., & Smith, S. D. (1990). Mechanisms associated with the variation in tenderness of meat from brahman and hereford cattle. *Journal of Animal Science*, 68, 4206-4220.
- Whipple, G., Koohmaraie, M., Dikeman, M. E., Crouse, J. D., Hunt, M. C., & Klemm, R. D. (1990). Evaluation of attributes that affect longissimus muscle tenderness in bos taurus and bos indicus cattle. *Journal of Animal Science*, 68, 2716-2728.
- Wiklund, E., Barnier, V. M. H., Smulders, F. J. M., Lundstrom, K., & Malmfors, G. (1997). Proteolysis and tenderisation in reindeer (*Rangifer tarandus tarandus* L.) Bull longissimus thoracis muscle of varying ultimate pH. *Meat Science*, 46, 33-43.
- Wiklund, E., Stevenson-Barry, J. M., Duncan, S. J., & Littlejohn, R. P. (2001). Electrical stimulation of red deer (*Cervus elaphus*) carcasses - Effects on rate of pH-decline, meat tenderness, colour stability and water-holding capacity. *Meat Science*, 59, 211-220.
- Wood, J. D. (1990). Consequences for meat quality of reducing carcass fatness. In: J. D. Wood, et al, *Reducing fat in meat animals* (pp. 397-399): Elsevier Applied Science.
- Wood, J. D., Enser, M., Fisher, A. V., Nute, G. R., Sheard, P. R., Richardson, R. I., Hughes, S. I., & Whittington, F. M. (2008). Fat deposition, fatty acid composition and meat quality: A review. *Meat Science*, 78(4), 343-358.
- Wright, D. J., & Wilding, P. (1984). Differential scanning calorimetric study of muscle and its proteins: Myosin and its subfragments. *Journal of the Science of Food and Agriculture*, 35, 357-372.
- Young, O. A. (1984). The biochemical basis of fibre type in bovine muscle. *Meat Science*, 11(2), 123-137.
- Young, O. A., & Gregory, N. G. (2001). Carcass Processing: Factors Affecting Quality. In: Y. H. Hui, W.-k. Nip, R. W. Rogers, & O. A. Young, *Meat Science and Applications*. New York: Marcel Dekker, Inc.

Appendix 1. Designed statistical analysis

Plot	animal	side	Trt	position on loin	ageing
1	1	L	control	1	3
2	1	L	control	2	2
3	1	L	control	3	1
4	1	R	stretched	1	3
5	1	R	stretched	2	2
6	1	R	stretched	3	1
7	2	L	stretched	1	2
8	2	L	stretched	2	1
9	2	L	stretched	3	3
10	2	R	control	1	2
11	2	R	control	2	1
12	2	R	control	3	3
13	3	L	control	1	1
14	3	L	control	2	3
15	3	L	control	3	2
16	3	R	stretched	1	1
17	3	R	stretched	2	3
18	3	R	stretched	3	2
19	4	L	stretched	1	1
20	4	L	stretched	2	3
21	4	L	stretched	3	2
22	4	R	control	1	1
23	4	R	control	2	3
24	4	R	control	3	2
25	5	L	stretched	1	2
26	5	L	stretched	2	1
27	5	L	stretched	3	3
28	5	R	control	1	2
29	5	R	control	2	1
30	5	R	control	3	3
31	6	L	control	1	3
32	6	L	control	2	2
33	6	L	control	3	1
34	6	R	stretched	1	3
35	6	R	stretched	2	2
36	6	R	stretched	3	1
37	7	L	stretched	1	2
38	7	L	stretched	2	1
39	7	L	stretched	3	3
40	7	R	control	1	2

41	7	R	control	2	1
42	7	R	control	3	3
43	8	L	control	1	1
44	8	L	control	2	3
45	8	L	control	3	2
46	8	R	stretched	1	1
47	8	R	stretched	2	3
48	8	R	stretched	3	2
49	9	L	stretched	1	3
50	9	L	stretched	2	2
51	9	L	stretched	3	1
52	9	R	control	1	3
53	9	R	control	2	2
54	9	R	control	3	1
55	10	L	control	1	1
56	10	L	control	2	2
57	10	L	control	3	3
58	10	R	stretched	1	1
59	10	R	stretched	2	2
60	10	R	stretched	3	3
61	11	L	control	1	2
62	11	L	control	2	3
63	11	L	control	3	1
64	11	R	stretched	1	2
65	11	R	stretched	2	3
66	11	R	stretched	3	1
67	12	L	stretched	1	3
68	12	L	stretched	2	1
69	12	L	stretched	3	2
70	12	R	control	1	3
71	12	R	control	2	1
72	12	R	control	3	2