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Effect of accelerated boning
of pork carcasses on fresh
meat quality and

Effect of accelerated boning of pork carcasses on fresh meat quality and tenderisation.

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Table 5.15 Purge at 4 and 10 days post slaughter and cooking loss at rigor, 1, 2, 4, 6, 8 and 10 days post slaughter in experiment 9 for lamb *M. longissimus thoracis et lumborum* after low voltage electrical stimulation for 50 seconds at 4 minutes post slaughter or no electrical stimulation and pelvic or Achilles suspension.

Table 5.16 Effects of rate of pH decline grouping on meat quality and tenderness of pork *M. longissimus thoracis et lumborum* from experiments 7 and 8.

Table 5.17 Effects of rate of pH decline grouping on meat quality and tenderness of lamb *M. longissimus thoracis et lumborum* from experiment 9.

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DEDICATION

This thesis is dedicated to the late Dr. Ron Parr who made all this possible in persuading me to become a “meaty”. Without his support, leadership, encouragement and continuous humour I would never have started let alone completed this study. To a remarkable man, thanks, I will never forget the help and friendship you gave.

STATEMENT OF AUTHORSHIP

The experiments described in this thesis were conducted at the Victorian Institute of Animal Science, Werribee between 1995-1998. All experimental procedures described in this dissertation were approved by the Animal Ethics Committee.

I declare that this thesis contains only my own work, except for material published by others where due reference is made. This work has not been submitted in any form for any other degree or diploma at any University.

ABSTRACT

Variations in pork tenderness, ageing and overall meat quality were investigated to determine optimal boning methods to overcome tenderness problems associated with pork. Accelerated boning, stunning method (carbon dioxide verses electrical), chilling conditions and low voltage electrical stimulation were investigated to determine the impacts of these conditions on eating quality of pork *longissimus*, particularly tenderness.

Accelerated boning and chilling at 0°C reduced pork tenderness, prevented ageing over 10 days of storage post slaughter and increased drip loss due to cold toughening. The toughness associated with accelerated boning and 0°C chilling was overcome by either temperature conditioning at 14°C and 4 days ageing or by the infusion of calcium chloride at 30 minutes or 6 hours post slaughter. Temperature conditioning at 14°C prevented cold toughening, prevented protein denaturation and increased the ageing rate. The use of calcium chloride increased drip loss and altered meat colour.

Various rates of ageing were observed to occur after conventional (cold) boning. The rate of pH decline post slaughter was shown to influence the tenderness of pork and lamb with a fast rate of pH decline post slaughter producing the most tender pork. Furthermore, tenderness was improved through the use of low voltage electrical stimulation, pelvic suspension chilling at 14°C relative to 2°C and storage post slaughter. These improvements in tenderness could all be attributed to the prevention of cold toughening as few differences were observed in protein denaturation and proteolytic activity.

ABBREVIATIONS

AB	accelerated boning
ach	Achilles suspension
ANOVA	analysis of variance
ATP	adenosine triphosphate
°C	Degrees Celsius
Ca	Calcium
CB	Conventional boning
CL	Carbon dioxide stunning, low voltage stimulation for 60 seconds
CN	Carbon dioxide stunning, no low voltage stimulation
CO ₂	carbon dioxide
CS	Carbon dioxide stunning, low voltage stimulation for 15 seconds
DFD	dark, firm dry
ELECT	electrical head to heart stunning
g	grams
hr	hour
kg	kilogram
LTL	<i>M. longissimus thoracis et lumborum</i>
LV	lumbar vertebra
LVES	low voltage electrical stimulation
MFI	myofibrillar fragmentation index
mm	millimetres
MRTC	Meat Research and Training Centre
nn	Homozygous positive for the halothane gene
nN	Heterozygous for the halothane gene
NN	Homozygous negative for the halothane gene
NS	no significant difference
pel	pelvic suspension
%	percentage
PSE	pale, soft, exudative
r	Correlation coefficient
RB	rigor boned
R ²	percentage variation accounted for
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis

se	Standard error
SED	standard error of the difference
SL	sarcomere length
s. protein	sarcoplasmic protein solubility
stim	low voltage electrical stimulation
susp	Suspension
t	Time
TV	Thoracic vertebra
μm	Micrometre
WBSF	Warner-Bratzler peak shear force

1. LITERATURE REVIEW.

The major objective for pork production is to produce a high quality product at an economical price. With the continual drive towards efficiency in a highly competitive market, modifications of processing methods are required to reduce costs without jeopardising quality. Recent changes in the pig meat processing industry have the potential for detrimental effects on eating quality, especially tenderness. The most important changes are the application of faster chilling rates, and the large reduction in the delay between slaughter and sale to consumers (Ouali, 1984). The impact that these changes to processing methods have on tenderness is a major concern to the Australian pig industry, with the present perception of Australian pork being 'tough and dry' in comparison to other meats (Bennett, 1997). The perceptions of 'tough and dry' pork have been attributed to a number of factors including the high incidence of pale, soft, exudative pork, inadequate intramuscular fat content, cold shortening, inadequate ageing and over cooking (Bennett, 1997).

Research in Australia and overseas has been conducted looking at pork quality, in particular pale, soft, exudative (PSE) and dry, firm, dark (DFD) pork (see D'Souza, 1998 for review). Many of the factors that contribute to these conditions have been identified such as pre-slaughter stress, mixing and delays in slaughter floor processing along with methods to reduce their occurrence (see D'Souza, 1998 for review). However, very little work has been conducted in Australia on the eating quality of pork, in particular tenderness, a characteristic of meat many believe to be the most important in ensuring consumer satisfaction (Bennett, 1997). Furthermore, some of the practices such as rapid chilling recommended to reduce PSE may be having a detrimental effect on the eating quality of pork (James *et al.*, 1983).

A recent study in Australia by Hofmeyr (1998) has revealed the need for investigation of pork tenderness. In a survey of Melbourne butchers and supermarkets, considerable variations in pork tenderness were identified, with 54% of samples being unacceptable to the consumer. The percentage unacceptable for each month ranged from 18 to 88% over the 6 months of the survey period (Hofmeyr, 1998). The cut off for acceptability was based on a Warner-Bratzler peak shear force value of 5 kg being the maximum for acceptability.

Furthermore, a survey conducted by the Australian Meat and Livestock Commission in 1995 (AMLC, 1995 cited in Bennett, 1997) showed that only 36% of consumers surveyed found pork delicious to eat while only 20% reported it to be tender and juicy and 31% said it was inclined to be dry making it the toughest and driest of all meats assessed. This compares to chicken with 71% saying it was delicious to eat, 51% saying it was normally tender and juicy and only 21% saying it was inclined to be dry (AMLC, 1995 cited in Bennett, 1997).

Although pork production has increased in Australia by 20% between 1990 and 1995, considerable competition across the meat industry has demanded improvements in production efficiencies. In 1996-97 overall meat consumption by Australians increased by 4.7% from the previous year, although overall pork consumption fell 3.8% (Aus-meat, 1998). Increased competition within the industry has stemmed from increases in pork imports from such countries as Canada along with increased competition from other meat sources. This increased competition has led to larger, leaner pork carcasses, the production of entire males and the implementation of more efficient processing procedures thereby increasing the slaughter rates (Trout, 1993).

Consumers demand an attractive, economically priced product with desirable colour, which is nutritious and healthy, tender, juicy and flavourful, with no fat or additives. At point of sale, consumers visually assess the product for size, shape, colour, lean to fat ratio, texture and cost (Chambers and Bowers, 1993; Kauffman and Marsh, 1987). Unless the product meets the consumers' visual approval, no sale will occur. Repeat purchase of the product is then dependent upon the consumers' response to aroma, taste, tenderness and juiciness of the product when they consume it in the home. Thus to optimise pork sales, methods of production are required to produce a consumer acceptable product at an economical price (Dalen, 1996).

Therefore, it is evident that further research is required to ensure the production of tender, juicy pork at an economical cost. This thesis examined pork eating quality, in particular tenderness, looking at various slaughtering and processing methods that may influence tenderness and overall pork quality. Furthermore, accelerated processing of pork carcasses was examined to determine its influence on tenderness

and overall pork quality. Thus methods to maximise eating quality while improving efficiency and reducing production costs could be determined.

In order to understand the factors that contribute to tenderness and overall quality of pork, an understanding of muscle structure and the proteins contributing to the variation in quality and tenderness is required. This is presented in the following literature review. Furthermore, a review of the different factors that influence pork tenderness and overall meat quality are presented.

1.1 MUSCLE COMPOSITION:

Muscles are the contractile organ of the body with a complex structure. Skeletal muscles contain approximately 75% water, 20% protein, variable amounts of lipids (3-15%) and carbohydrates and a small amount of soluble organic compounds (Jiang, 1995). Muscle is comprised of bundles of fibres (muscle cells) sheathed in connective tissue called the perimysium (Figure 1.1). Each fibre is a giant multinucleated cell and may attain a length of 34 cm (Lawrie, 1991). Within each fibre are thousands of parallel myofibrils, the rod like organelles responsible for contraction, which occupy up to 80% of its volume. The myofibrils are responsible for the striated appearance of the muscle (Figure 1.2). The myofibrils are bathed in the intracellular fluid known as the sarcoplasm, containing many enzymes such as those responsible for glycolytic metabolism (Bechtel, 1986).

Each muscle fibre is surrounded by its own connective tissue, the endomysium and by its own membrane, the sarcolemma (Figures 1.1 and 1.2). The sarcolemma periodically invaginates into the fibre to form T-tubules which transmit the action potential into the region of the contractile machinery where it stimulates the sarcoplasmic reticulum to release calcium (Bechtel, 1986). At the cessation of the stimulus, calcium is actively pumped back into the sarcoplasmic reticulum, which lies along each side of the T-tubules (Figure 1.2).

Alternating light and dark bands are clearly visible along the myofibrils and are formed by structural proteins. The myofibrils consist of contractile filaments in a regular array of sarcomeres, consisting of thick myosin filaments, thin actin filaments and associated proteins (mainly troponin and tropomyosin) (Figure 1.3). The light

and dark bands that are visible under the microscope arise from the diffraction transmittance of light through the thick myosin and thin actin filaments arising from the inter-digitation of filaments. Thin filaments emanate from the Z-line and are in the I band while the thick and thin filaments make up the A band. The thin filaments extend into the A band while the H zone is the non overlapping region of the actin and myosin. Each sarcomere extends from one Z line to the next with the Z lines maintaining the spacing between the thin filaments (Bechtel, 1986). Other myofibril proteins are responsible for maintaining the structural integrity of muscles via inter-myofibril linkages (desmin and vinculin) and intra-myofibril linkages (titin and nebulin) (Koochmaraie *et al.*, 1995).

The two main muscle proteins by weight are actin and myosin. Myosin is the main protein present in thick filaments accounting for 43% (w/w) of total myofibrillar proteins (Yates and Greaser, 1983). A myosin monomer is composed of a rod shape with 2 globular regions known as myosin heads attached at one end and the thick filament is made up of many myosin monomers (Pearson and Young, 1989). The myosin heads contain binding sites for actin, adenosine triphosphate (ATP) and the enzyme site for hydrolysis of ATP. Actin is the main protein of the thin filament, accounting for 22% (w/w) of total myofibrillar proteins (Yates and Greaser, 1983). Actin can exist in two forms, either as a small globular unit known as G-actin or as an aggregation of the globular units forming a double chain known as F-actin (Lawrie, 1991). Actin is connected to the z line at one end and contains binding sites for tropomyosin and troponin, proteins involved in preventing actin and myosin from binding during the resting state.

Contraction of living muscle upon stimulation results from a complex series of biochemical reactions. Living muscle contracts through the overlapping of the actin and myosin filaments within myofibrils, upon nervous stimulation. In resting muscles, binding between actin and myosin is prevented by the binding of tropomyosin and troponin to actin. Upon stimulation due to ATP depletion, calcium ions are released from the sarcoplasmic reticulum thus activating the glycolytic enzymes to produce ATP that provides the energy required for muscle contraction. The myosin binding site on actin is exposed by calcium binding to troponin-C which moves troponin T aside so that myosin can bind with actin to form the actomyosin complex. The charged myosin which is tightly attached to actin, swivels and causes the filaments to

slide (Jiang, 1995). Calcium ions are actively pumped back into the sarcoplasmic reticulum provided sufficient ATP is available, causing the actomyosin complex to break and the muscle to relax to its original state.

Approximately 5.5% of the total mass of skeletal muscle (Asghar and Bhatti, 1987) and 30-35% of total muscle protein (Pearson and Young, 1989) is made up of sarcoplasmic proteins. Sarcoplasmic proteins, consisting of over 100 different proteins, are present in the sarcoplasm and are involved in cellular metabolism - glycolytic enzymes, creatine kinase, myoglobin, enzymes for protein synthesis and degradation, fatty acid oxidation, electron transport, phosphorylation and other enzyme systems (Pearson and Young, 1989; King and Macfarlane, 1987). All constituents of the glycolytic cycle are contained within the sarcoplasm and are responsible for energy production and the formation of lactic acid during the onset of rigor mortis (Pearson and Young, 1989). The glycolytic enzymes are reversibly bound to the myofibrillar proteins. Changes to the proportions of bound enzymes affects the flux through the glycolytic pathway. Furthermore, the sarcoplasmic proteins include the calpain proteolytic enzymes involved in protein degradation post slaughter.

1.1.1 Fibre types:

Muscles differ greatly in their rates of physiological reactions reflecting the properties of the predominant fibre types and this contributes to the differences in tenderness and meat quality that occur in muscle post rigor. Muscles can contain up to four major functionally specialised groups of fibres: (1) slow twitch oxidative (type I), (2) fast twitch oxidative glycolytic (type IIA), (3) fast twitch glycolytic (type IIB) and (4) intermediate (type IIC) (Pearson and Young, 1989). These fibres contain qualitatively different contractile proteins, different proteinase and inhibitor systems as well as varying amounts of myoglobin and oxidative and glycolytic enzymes (Pearson and Young, 1989). Due to varying physiological functions of muscles, each muscle contains a unique proportion of muscle fibre types (Jiang, 1995). Due to this variability in fibre type composition, a heterogeneous pattern of post slaughter behaviour between and within muscles and adjacent fibres can be expected (Jiang, 1995). Different muscle fibre types will produce differences in eating quality properties between and within muscles and affect properties such as meat colour, juiciness, flavour and tenderness (Monin and Ouali, 1991).

The majority of muscles are made up of a mixture of muscle fibre types, influencing meat quality characteristics post slaughter. The variation in proportions of the different muscle fibre types between muscles influences the rate of pH fall and the response to electrical stimulation (Devine *et al.*, 1984) for example muscles with high content of fast twitch glycolytic fibres are more responsive to electrical stimulation and a faster rate of pH decline. The *M. longissimus thoracis et lumborum* (LTL) is termed a white muscle in the pig due to the high level of fast twitch glycolytic fibres, consisting of approximately 84% fast twitch glycolytic, 10% fast twitch oxidative glycolytic and 6% slow twitch oxidative (Kiessling and Hansson, 1983). Hunt and Hedrick (1977) reported the pork LTL muscle contained only 46% fast twitch glycolytic, 25% fast twitch oxidative glycolytic and 29% slow twitch oxidative muscle fibres. Therefore, due to the high content of fast twitch glycolytic fibres in the loin muscle, the susceptibility of this muscle to the PSE condition following a rapid pH decline as a result of electrical stimulation, the presence of the halothane gene, or preslaughter stress is increased.

1.2 CONVERSION OF MUSCLE TO MEAT:

The conversion from muscle to meat is a complex process. The process that initiates the conversion of muscle to meat is the cessation of blood circulation resulting in the depletion of muscle oxygen and a build up of cellular waste products (Marsh, 1981). This results in local metabolism continuing anaerobically (Judge *et al.*, 1989), reducing the pH and energy levels of the muscle. As a result of the fall in pH and the lack of ATP, rigor mortis develops, osmotic pressure rises and proteins denature (Lawrie, 1992). Once rigor mortis is complete, exudation, discolouration, rancidity, lysis of proteins and microbial growth proceed and their rate is influenced by muscle temperature (Lawrie, 1992). Alterations to any of the conditions during rigor onset can alter the ultimate meat quality (Bendall, 1973).

The time required for rigor mortis to set in is dependent upon biochemical and physical characteristics of the muscle. The onset and extent of pH fall are biochemically characterised by the content of energy rich compounds including ATP, creatine phosphate and glycogen and is initiated by the decrease in oxygen supply (Judge *et al.*, 1989). Furthermore, the activities of ATPase and glycolytic enzymes in

the muscle play an important role in determining the rate of rigor onset (Jiang, 1995). The rate of conversion of muscle to meat also depends upon species, breed, pre-slaughter conditions, the muscle temperature, relative humidity, the level and activity of indigenous proteolytic enzymes, initial muscle glycogen content and muscle type (Judge *et al.*, 1989).

Changes in muscle extensibility parallel changes in ATP content (Judge *et al.*, 1989). In pre-rigor muscle, ATP prevents the cross-bridging between the thick and thin filaments but with a falling level of ATP, progressive cross bridging occurs (Marsh, 1981). Furthermore, when the concentration of ATP becomes very low, calcium ions released from the sarcoplasmic reticulum can no longer be reabsorbed by the ATP - calcium pumps. The resulting high calcium ion concentration activates the troponin-tropomyosin system which unblocks the binding site for myosin heads to actin filaments, resulting in the myosin and actin filaments binding together, forming the rigid and in-extensible state, known as rigor mortis (van Laack, 1989).

The onset of rigor is characterised by a reduction in muscle pH and a depletion of ATP. The oxygen in the muscle at death is quickly depleted causing all oxidative metabolism to cease and anaerobic glycolysis continues (Judge *et al.*, 1989). Glycogen continues to breakdown to pyruvate before conversion to lactate, accumulating in the tissue and causing a pH decline from around 7.0 at death to roughly 5.5 after rigor is complete (Marsh, 1981). This generation of lactate from pyruvate subsequently slows the regeneration of ATP as it is the conversion of pyruvate to CO₂ under aerobic conditions that is most efficient at regeneration of ATP. The anaerobic glycolytic process produces about 10% of the ATP that can be produced under aerobic metabolism. Due to the regeneration of ATP from the high energy compound creatine phosphate (CP) by the enzyme creatine phosphokinase, ATP levels are not seen to fall for several hours post slaughter at which time all of the creatine phosphate is exhausted. When the pH falls to around pH 6.0, the ATP level falls rapidly to a value which is insufficient to keep the actin and myosin apart thus they unite irreversibly to form inextensible actomyosin, producing the stiffness of rigor mortis. This reduction in ATP causes the rapid phase of rigor onset (Marsh, 1981) and rigor has normally begun by the time muscle pH of 5.9 has been reached (Hamm, 1981).

The rate of pH decrease in muscle post slaughter is related to the contractile and metabolic type of muscle, preslaughter stress, temperature of chilling, muscle temperature at slaughter and species. The rate and extent of pH fall post slaughter depends primarily on the levels of glycogen and phosphate compounds at death, the rate of ATP turnover and the buffering capacity of the muscle tissue (Jiang, 1995). Glycolysis proceeds until the glycogen stores are depleted or the pH is lowered to less than 5.4 where glycolytic enzyme inhibition occurs which prevents further glycolysis. Normal pH decline patterns seen in pork muscle are from a starting pH of 7.4 in living muscle declining to about 5.6 - 5.7 within 6-8 hours post slaughter and then to an ultimate pH of 5.3 - 5.7 by 24 hours post slaughter (Judge *et al.*, 1989).

There are a number of factors that affect the rate and extent of post slaughter glycolysis which has important effects on the subsequent meat quality. These factors are discussed in section 1.6 in this literature review.

1.3 MEAT QUALITY CHARACTERISTICS:

Consumers demand pork with high meat quality characteristics. The major pork quality characteristics that are easily assessed by the consumer are colour and the level of drip loss. Both these factors are influenced by the rate and extent of post slaughter glycolysis and thus are influenced by the processing method employed. Furthermore, these characteristics are the basis for classifications of PSE, DFD and 'normal' pork used in the pork industry.

1.3.1 Pale, soft, exudative pork

Pale, soft, exudative (PSE) pork is a major problem in the Australian pork industry with 51% of Australian pork classified as PSE (Eldridge *et al.*, 1995) at a cost of \$24 million per year (Whan, 1993) resulting from a rapid pH decline post slaughter. PSE is classified as meat that has a surface lightness (CIE-L*) > 50 and a drip loss > 5% (Warner *et al.*, 1996). Rapid glycolysis post slaughter leads to a rapid pH decline while the muscle temperature is still high and is often a result of pre-slaughter stress. This decline in pH can cause rigor development to be reached within 15 minutes post slaughter and the ultimate lactate concentration to be reached by one hour (Greaser, 1986). Protein denaturation directly causes PSE pork and is induced by a slow temperature decrease and rapid pH decline post slaughter (Honikel, 1986) as a result

of such factors as the presence of the halothane gene, pre-slaughter stress, electrical stimulation and slow chilling.

The increased protein denaturation as a result of the fast pH decline post slaughter at high temperatures that is observed in PSE pork results in unacceptable meat quality characteristics. The rapid pH decline induces protein denaturation causing the reduction in the water holding capacity of the muscles (Penny, 1969). The loss of water is further accelerated by the low ultimate pH of PSE pork, approaching the myofibrillar proteins isoelectric point thereby reducing the available protein charges available to bind water (Judge *et al.*, 1989; Kauffman and Marsh, 1987). Thus the high amount of free water located between the muscle cells reflects a greater amount of light due to the reflective properties of water thereby reducing the colour intensity (Judge *et al.*, 1989). Furthermore, denaturation of the proteins due to the rapid pH decline results in a loss of protein solubility, loss of water and a reduction in water binding capacity resulting in lower processing yields, increased cooking loss and a reduction in juiciness (Judge *et al.*, 1989). The reduced level of extractable myofibrillar proteins and lower solubility of sarcoplasmic proteins of PSE pork, has a profound effect on the usefulness of PSE muscle in processed products (Bendall and Wismer-Pedersen, 1962).

There are a number of ways to reduce PSE. PSE can be prevented by reducing pre-slaughter stress (D'Souza *et al.*, 1998a; Grandin, 1980), reducing halothane gene carrier pigs (Channon *et al.*, 1998; Murray *et al.*, 1989) and rapid chilling (Iversen *et al.*, 1995). Minimisation of pre-slaughter stress and fast chilling can provide the possibility of preventing PSE meat when the presence of the halothane gene is not a component. Furthermore, rapid chilling within the first few hours post slaughter diminishes the exudative and pale characteristics of these muscles (Honikel, 1986). Removal of muscles from the carcass pre-rigor has the potential of diminishing mild PSE meat and thereby improving the quality and economics due to the ease of chilling cuts versus carcasses.

1.3.2 Dark, firm, dry pork

Another quality problem in the Australian pork industry is dark, firm, dry (DFD) pork. DFD results from low glycogen reserves in the muscle prior to slaughter which

reduces the normal post slaughter decrease in muscle pH. Slow twitch oxidative muscles are more prone to DFD than fast twitch glycolytic muscles due to their naturally lower glycogen reserves. The dark colour of DFD pork can be attributed to a higher proportion of deoxygenated myoglobin because at a higher ultimate pH, oxygen uptake and utilisation is greater on the eat surface after cutting and less oxymyoglobin is formed due to slower enzyme reactions(Fox, 1987). The dry surface appearance can be attributed to the water being tightly bound to the proteins as the proteins are highly charged due to the high ultimate pH of DFD pork. This pH is much higher than the myofibrillar protein isoelectric point (Judge *et al.*, 1989). DFD pork is defined as having a surface lightness < 42, a drip loss < 5% and an ultimate pH > 6.0 (Warner *et al.*, 1996).

DFD pork results from low levels of muscle glycogen at slaughter. Reduced glycogen stores are often associated with long lairage times (longer than 24 hours) especially when mixing of entire males occurs (Gregory, 1990), prolonged periods of transportation, extended time off feed, fighting, prolonged stress and over-activity (Barton-Gade, 1991).

The main problems associated with DFD pork is its short shelf life as it is more susceptible to microbial spoilage than normal pork as the absence of glucose at the surface allows spoilage microflora to attack proteins (Newton and Gill, 1981) and consumer acceptance is reduced due to the darker colour. Although DFD pork is associated with a shorter shelf life, it is generally more tender after cooking (Kauffman and Marsh, 1987).

1.3.3 Meat Colour

Meat colour is one of the most important factors influencing retail sales as it is easily assessed by the consumer. Furthermore, colour is greatly influenced by the rate and extent of glycolysis. Meat colour variation results from the myoglobin concentration of the muscle and changes in chemical state of myoglobin. The extent of colour variation is influenced by the ultimate pH, the water holding capacity (Judge *et al.*, 1989), the myofibrillar matrix (the interaction of actin and myosin influencing the level of muscle contraction) and the level of protein denaturation (Ledward, 1995). Much

of the variability in meat colour of normal pH carcasses is accounted for by the variation in myoglobin quantity in muscle (Warner, 1988).

Myoglobin contributes 80-90% of the total pigment in muscle tissue of a well bled carcass (Judge *et al.*, 1989). The chemical forms of myoglobin affecting the colour of fresh meat are the purplish red myoglobin, the bright red oxymyoglobin and the dull brown metmyoglobin (Tinbergen, 1975). The three forms of myoglobin are inter-convertible. The oxygenation of myoglobin to oxymyoglobin by the binding of molecular oxygen occurs after the meat has been cut and exposed to air and is called the "blooming" of meat.

There are a number of factors that influence meat colour. Meat of a high ultimate pH is generally associated with a darker colour (Ledward, 1985). Advances in the age of an animal generally results in a higher concentration of myoglobin giving the meat a redder and darker colour. Accelerated processed meat has a darker colour (van Laack, 1989; Shaw and Powell, 1995) due to a higher ultimate pH as a result of faster chilling rates (Bowles *et al.*, 1983), increased water holding capacity (Kastner, 1982), reduced protein denaturation (Ledward, 1985) or a more densely packed myofibrillar protein matrix due to greater muscle shortening (Judge *et al.*, 1989). Electrical stimulation has been reported to improve beef muscle colour resulting in a brighter, more attractive appearance (Aalhus *et al.*, 1994). This improvement is believed to be caused by the increased rate of glycolysis resulting in a faster rate of pH decline with the lower pH increasing reflectance (Pearson and Dutson, 1985). It is believed that as more protein will be denatured following stimulation as a result of lower pH, the amount of free water at the surface increases thereby increasing reflectance. Also the tearing apart of muscle fibres induced by electrical stimulation may promote improved colour (Pearson and Dutson, 1985).

1.3.4 Water holding Capacity

Water holding capacity of meat is an important quality characteristic as it affects the appearance of meat before cooking, its behaviour during cooking and its juiciness during consumption (van Laack, 1989). Furthermore, it is important to the retail industry due to drip loss reducing final weight, reduced yields during cooking and increased problems in the processing industry.

1.3.4.1 *Rigor mortis*

As muscle goes into rigor the water holding capacity of the muscle is reduced (Hamm, 1986). Muscles consist of approximately 75% water (Jiang, 1995) which is primarily bound by myofibrillar proteins with varying forces of interaction (Tyszkiewicz *et al.*, 1995). The immobilisation of water is related to spatial arrangements of the proteins and the availability of intermolecular spaces between the myofibrils (Offer and Knight, 1988) and most of the alterations in water holding capacity are due to changes in the physical configuration of the myofibrillar proteins. Muscle proteins increase in density with rigor mortis due to the binding of actin and myosin causing muscle contraction which reduces sarcomere length, thus the ability of the muscles to hold onto water is reduced due to the reduction in myofibrillar lattice space resulting in a loss of moisture. This reduction in water holding capacity is further enhanced due to the water holding properties of actomyosin being less than that of myosin and actin alone (Offer and Knight, 1989).

1.3.4.2 *Protein denaturation*

In addition to the reduction during rigor mortis, the water holding capacity of meat is also reduced by the denaturation of both sarcoplasmic and myofibrillar proteins which occurs during rigor onset (Scopes, 1964). Rapid pH decline while temperatures are still high denatures muscle proteins which results in a reduction of the water holding capacity. Denaturation of muscle proteins pre-rigor can be assessed by several methods including: solubility of myofibrillar proteins (Wismer-Pederson, 1959; Bendall and Wismer-Pederson, 1962), solubility of sarcoplasmic proteins (Sayre and Briskey, 1963), activity of myosin and myofibrillar ATPase (Greaser *et al.*, 1969), activity of some sarcoplasmic proteins (Fischer *et al.*, 1979) and precipitation of sarcoplasmic proteins onto myofibrils (Fischer *et al.*, 1977). With increased muscle protein denaturation, a reduction in protein solubility, a reduction in ATPase activity and an increased precipitation of sarcoplasmic proteins will be observed with the reduction in extractability of myofibrillar proteins and myofibrillar ATPase activity being the easiest to assess.

Myosin is most susceptible to denaturation at the ATPase active site and this can result in conformation changes prior to binding with actin at rigor. Changes in pH are expected to affect the ATPase active site of myosin and the overall conformation of

myosin (Offer, 1991). Denaturation of the active site of myosin is reflected in a reduction in ATPase activity while conformation changes are reflected in altered solubility. Myosin is susceptible to denaturation prior to formation of the rigor complex between actin and myosin at which time myosin can no longer be denatured (Offer, 1991). Thus changes in pH decline rate will influence the rate of myosin denaturation due to changes in the rate of actomyosin formation (van Laack *et al.*, 1994).

Care must be taken during carcass processing to prevent conditions that will promote protein denaturation. When a rapid rate of reduction of muscle pH occurs post slaughter while carcass temperature is still relatively high, myofibrillar and sarcoplasmic proteins will be denatured, reducing the water holding capacity of the muscle and giving the muscle a paler colour (Wismer-Pedersen, 1959; Bendall and Wismer-Pedersen, 1962; Offer and Knight, 1988). It has been found that cold temperatures and high relative humidity are generally beneficial in reducing protein denaturation due to acceleration of temperature decline post slaughter (Pearson, 1986) thereby preventing PSE. Cold temperatures also retard bacterial degradation and slow the action of indigenous muscle enzymes while high relative humidity at low temperatures accelerates the rate of heat transfer from the carcass, thus speeding up the chilling process (Pearson, 1986).

1.4 MEAT TENDERNESS:

Tenderness can be defined as the ease with which a product is penetrated, fractured and broken down during mastication and can be related to several factors. A long sarcomere length predisposes muscles to greater tenderness and shortening of muscles induces toughening (Davis *et al.*, 1979; Marsh and Leet, 1966; Herring *et al.*, 1965). Tenderness is also influenced by the content and quality of connective tissue and depends upon the age, sex, muscle type and species with pork tenderising faster than lamb and beef (Ouali, 1990). Pork is more tender than beef and lamb due mainly to the faster glycolytic rate of pork relative to both beef and lamb (Ouali, 1990) and differences in the level of calpains, calpastatin and cooling rates post slaughter (Dransfield, 1992d) with pork having a slower temperature decline than lamb and a faster temperature decline than beef.

In the pre-rigor condition, muscle is tender and becomes tougher as rigor mortis sets in due to linkage of the actin and myosin and then becomes more tender with storage (Judge *et al.*, 1989). Tenderness cannot be measured following cooking in the pre-rigor period because of the inability to prepare pre-rigor muscle without shortening occurring (Dransfield, 1994). The extent of decrease in toughness with ageing seems to be related to the extent of the increase in toughness during the first 24 hours (Dransfield, 1994) due to alterations in sarcomere length and initial activity of the proteolytic enzymes. It has been postulated that muscle tenderisation results from the disappearance of z-discs, a loss of the transversal alignment of z-disks, m lines and other contractile elements (see Ouali, 1990), dissociation of actomyosin complex (Takahashi *et al.*, 1987, 1985a, 1985b), destruction of titin and breakdown of collagen. The mechanisms involved in tenderisation are both enzymatic and physiochemical in nature (Jiang, 1995).

The improvement in tenderness due to ageing is largely due to proteolysis (Geesink, 1993). Proteolysis results in a loss of structural integrity of the muscle (Geesink, 1993) with progressive fracturing of the thin filament attachments to the z disc but not at the cross bridges (Etherington, 1984). The differences in the rate and extent of post slaughter proteolysis are the major sources of variation in meat tenderness (Koohmaraie *et al.*, 1995). The rate and extent of proteolysis is influenced by such factors as rate of glycolysis, rate of pH decline, degree of sarcomere shortening, osmolarity of muscle cells, temperature and genetic factors inherent to the animal (Goll *et al.*, 1995).

During post mortem ageing, the susceptibility of muscle to fragment is increased and the degree of fragmentation is temperature dependent (Olson *et al.*, 1976) due to the kinetic temperature requirements of the proteolytic enzymes. Fibres fragment at the I band or separate into fibre segments and single myofibrils (Steen *et al.*, 1997). The size of the fibre pieces becomes progressively smaller with time post slaughter. Studies by Olson *et al.* (1976) showed that at 1 day post slaughter the myofibrils are much longer but as post slaughter time increases, myofibrils become shorter and more fragmented. The structural region of the z-disc seems to be the site of this myofibril fragmentation.

A component of meat toughness is derived from the connective tissue, particularly that of the perimysium (Etherington, 1984). This element of toughness is dependent upon the collagen cross linking and changes in stability of the links within the muscle with the age of the animal as the individual fibres become increasingly cross linked with age (Dutson, 1972). When meat is cooked, the collagen fibres contract, increasing the muscle fibre density per unit area thereby increasing toughness particularly in muscles with a high collagen content (Harper *et al.*, 1995). The collagen can be hydrolysed by a number of proteinases, including cathepsins B + L, via cleavage in the telopeptide region (Etherington, 1980), releasing the cross link attachments between neighbouring collagen molecules (Etherington 1984).

The muscle fibre type composition contributes to the rate of tenderisation during post slaughter storage (Abott *et al.*, 1977). Ageing occurs more rapidly in fast twitch glycolytic muscles than in slow twitch oxidative muscles (Abott *et al.*, 1977). This difference in ageing rate is due to differing susceptibilities of the proteins to proteolysis, varying levels of *m*-calpain and the calpain inhibitor calpastatin and changing ionic strength (Klont *et al.*, 1998). In addition, slow twitch oxidative fibres are less affected by a rise in ionic strength than fast twitch glycolytic fibres because of the lower solubility of their proteins (Geesink *et al.*, 1995).

1.5 MOLECULAR CHANGES OCCURRING DURING AGEING OF MEAT:

Meat becomes more tender during storage post-rigor. On average, 50% of the tenderisation has been observed to occur by 2 days post slaughter for pork and by 4.2 days for beef, veal and rabbit (Dransfield *et al.*, 1980-81). Eighty percent of tenderisation has been reported in pork by about 5 days and at least 2 weeks for beef (Etherington *et al.*, 1987).

The full tenderness potential after ageing is only obtained if cold shortening is avoided (Iversen *et al.*, 1995; Moller and Jensen, 1993). Studies in New Zealand showed that the extent of ageing decreased with increased muscle shortening with shortening of 40% resulting in no subsequent ageing with storage post slaughter (Marsh and Leet, 1966). The lack of ageing is caused by structural changes which prevent proteolysis thereby preventing tenderisation (Dransfield, 1994). However,

other studies have found cold shortened muscle to age in a similar manner to muscles which have not cold shortened (Koochmaraie *et al.*, 1998).

Changes with ageing have been observed to occur in the myofibrils, sarcoplasmic proteins, actomyosin complex and connective tissue (Ouali, 1990). Most studies looking at ageing have concentrated on the myofibrillar fragmentation that occurs in the region of the z disc as such fragmentation is correlated to loss of toughness. Major molecular and morphological alterations that have been observed to occur in meat during storage include:

1. z disc weakening and/or degradation leading to fragmentation of myofibrils,
2. alterations of actomyosin interaction, but the proteins myosin and actin themselves are not altered,
3. degradation of troponin-T to a 30 kDalton component, an essential regulatory protein located on the thin filament and responsible together with troponins I and C, and tropomyosin for the calcium dependence of the myofibrillar ATPase during contractions,
4. degradation of desmin leading to longitudinal fragmentation of myofibrils probably through disruption of transverse cross-linking between myofibrils,
5. degradation of titin which connects myosin filaments from the M-line to the z-disc,
6. degradation of nebulin located in the I bands of myofibrils, and
7. appearance of a 95 kDalton polypeptide, the origin of which is unknown (Koochmaraie, 1994).

Endogenous muscle proteolytic enzymes are the major determinants of meat tenderisation during ageing in the absence of cold shortening (Pearson and Young, 1989). In order to be considered as possible proteolytic enzymes involved in meat tenderisation a proteinase needs to be located within the skeletal muscle cell, have access to the myofibrils, and have the ability to degrade the same proteins that are degraded during post slaughter storage (Koochmaraie, 1994). Calpains μ and m , and the cathepsins B, L and D all have these characteristics (Pearson, 1986).

It is generally accepted that tenderisation results from proteolysis by endogenous enzymes but major problems have existed in identifying the exact enzymes. The major problem in identifying the specific enzymes has been that enzyme activity cannot be measured *in situ* since the enzymes depend on local *in situ* concentrations

of co-factors and inhibitors (Etherington, 1987). As the exact enzyme or enzymes causing the disruption of the myofibrils has not been identified, the most probable explanation is that the enzymes are acting synergistically (Etherington, 1987).

Specific actions of each enzyme involved in tenderisation have not been identified but overall the enzymes hydrolyse components of muscle fibres (Alarcon-Rajo and Dransfield, 1995). The hydrolysis of muscle fibre components results in disruption of muscle integrity and an improvement in tenderness (Alarcon-Rajo and Dransfield, 1995). When activity of the tenderising enzymes is inhibited, ageing can be prevented (Alarcon-Rajo and Dransfield, 1995). It has been demonstrated that cysteine proteinase inhibitors (calpain inhibitor) are more effective than serine (cathepsin B+L inhibitor) or aspartate inhibitors (cathepsin D inhibitor) while a combination of both cysteine and aspartate inhibitors was the most effective in preventing ageing (Alarcon-Rajo and Dransfield, 1995). As pepstatin inhibition of cathepsin D is not seen until day six post slaughter, it is suggested that cathepsin D plays a role in the later stages of ageing (Alarcon-Rajo and Dransfield, 1995).

1.5.1 Calpains:

The calpain enzymes are believed by many to be the most important of the enzymes involved in meat tenderness. Two calpains have been identified - *u* calpain (or calpain I), an enzyme requiring low calcium and showing one-half maximal activation at 1-30 μ M calcium and *m* calpain (or calpain II), an enzyme requiring high calcium and showing one-half maximal activation at 250-750 μ M calcium (Dayton *et al.*, 1981, Koohmaraie *et al.*, 1995). The enzymes are optimally active at pH 7-7.5 but retain significant activity at pH values as low as 5.5 (Geesink, 1993).

The calpain enzymes require calcium for activation. Calcium binds to the enzyme and induces autolysis of the enzyme which activates it (Koohmaraie *et al.*, 1995; Dransfield, 1994). Further autolysis results in loss of activity (Geesink, 1993). μ -Calpain is activated first, at low calcium ion concentrations and then *m*-calpain is activated as the concentration of calcium increases. During the conversion of muscle to meat, there is enough calcium ions to activate 100% of μ -calpain but only about 30% of *m*-calpain (Dransfield, 1994), thus the addition of exogenous calcium ions

results in an increased activation of *m*-calpain inducing more rapid and extensive tenderisation (Koohmaraie *et al.*, 1995).

The activity of calpain is influenced by many factors. The factors influencing the activity of *u*-calpain include the ultimate pH, initial calpastatin level and the post slaughter decline of calpastatin, rate of pH and temperature fall and inactivation of *u*-calpain through autolysis (Dransfield, 1994; Geesink, 1993; Ouali and Talmant, 1990). Activation of μ -calpain in beef begins at around pH 6.3 as the calcium concentration is sufficient for activation at this pH while *m*-calpain becomes activated at approximately 16 hours post slaughter following rigor onset when calcium concentrations are high enough (Dransfield, 1994). Due to the high ultimate pH of DFD pork it has been suggested that improvements in tenderness observed in DFD pork can be attributed to the enhanced activity of μ -calpain as the muscle stays at the pH which is optimal for the protease (Geesink, 1993).

The calpain enzymes are inhibited by calpastatin, reducing the activity of these enzymes (Ouali and Talmant, 1990; Geesink, 1993). The relative proportion of calpain to calpastatin influences the activity of the enzymes and then the rate of proteolysis of myofibrillar proteins and thus tenderisation (Ouali and Talmant, 1990; Geesink, 1993).

Proteolysis of muscle proteins by calpain is influenced by the stability of the enzymes and the amount of the enzyme and the enzyme inhibitor present. The amount and activity of calpains (due to the presence of calpastatin) in the various muscles varies according to animal production (eg entire males versus castrates, *Bos indicus* versus *Bos taurus*), rate of rigor development, storage conditions of the meat (Dransfield, 1994) and muscle type (Ouali, 1990). When activated, both calpains are unstable and become progressively less active with storage (Dransfield, 1994). Autolysis eventually results in loss of activity of the enzyme (Geesink, 1993).

Calpains have been reported to contribute to over 90% of tenderisation via degradation of myofibrillar proteins (Goll *et al.*, 1991; Geesink, 1993). Degradation of both titin and nebulin at sites in the I band, near the z disc have been observed to occur due to calpains (Goll *et al.*, 1995). Some researchers believe that calpain is the only proteolytic system that has all of the characteristics that are necessary for

bringing about the post slaughter changes that result in meat tenderisation (Koohmaraie, 1994). However the rates of protein degradation and tenderisation do not parallel the changes in calpain activity, indicating that calpain alone cannot account for the tenderisation process (Barnier *et al.*, 1993).

The involvement of calpains in tenderisation is supported by several findings. As ageing progresses, the major ultrastructural changes seen are the disappearance of the Z discs and a loss of transversal alignment of Z-discs, M-lines and other contractile elements (Ouali, 1990). The fractures along the Z line have been shown to be caused by calpains. The involvement of calpains in tenderisation is supported by:

1. the ultrastructural degradation of postmortem myofibrils being similar to that of myofibrils treated with calpain,
2. postmortem myofibrillar proteins untreated or treated with calpain have similar electrophoretic degradation patterns,
3. the z disc is extremely susceptible to calpain catalysed hydrolysis, and
4. the higher the level of calpains in muscle the faster the rate of postmortem tenderisation (Dayton *et al.*, 1981).

1.5.2 Cathepsins:

Another group of enzymes thought to be responsible for tenderisation are the cathepsins - B, D, H and L (Pearson and Young, 1989). Most of the tenderisation has been attributed to the action of cathepsins D, B and L, cathepsin H being thought to cause little structural alterations (Ouali, 1990). Cathepsins are lysosomal proteases which appear to be released during the ageing process due to lysosome dissolution (Chambers *et al.*, 1994). Cathepsins are optimally active at acid pH (pH<6) and high temperatures (maximum activity at 45°C) (Zeece and Katoh, 1989).

Cathepsins have been shown to degrade several myofibrillar proteins. Degradation of titin and nebulin have been observed after treatment of myofibrils with cathepsins (Zeece and Katoh, 1989) along with degradation of myosin heavy chain and actin (Asghar and Bhatti, 1987; Zeece *et al.*, 1986). Furthermore changes to the myosin heavy chain and actin resulting in a reduction in A band width (Geesink, 1993), the

formation of a cleared central region in the A band and dislocation of the z line (Zeece and Katoh, 1989) have also been observed due to cathepsin activity.

The effect of the cathepsin enzymes on myofibrillar structures is obviously influenced by the release of the enzymes from the lysosomes as this would determine their proximity to the substrate (Chambers *et al.*, 1994). Thus anything that affects the release of these enzymes from the lysosomes will influence their action on myofibrils. Conditions occurring post slaughter such as low pH, high temperature, high calcium concentration and low ATP concentrations negatively affect the stability of lysosomal membranes, thus enhancing the release of these enzymes. The stability of lysosomal membranes is generally reduced by the lowering of pH. This factor does not seem to be so important post slaughter as the majority of lysosomes are damaged before the ultimate pH is reached thus the other labialising factors play more important roles (Kas *et al.*, 1983).

Lysosomal membranes have been shown to degrade during ageing with an increase in free enzyme activities being observed with time (Chambers *et al.*, 1994). The specific activity of β -glucuronidase, a lysosomal enzyme used as an indicator for lysosomal breakdown, has been found by Chambers *et al.* (1994) to be highest in the microsomal fraction isolated from bovine *sternomandibularis* muscle just after slaughter. The microsomal fraction contains the enzymes still located in the lysosomes rather than the enzymes which have been released. They found that the level of activity for the microsomal fraction decreased significantly after seven days and again after 14 days ageing when it was slightly less than half of the value at death. Over the 14 days a significant increase in β -glucuronidase activity in the suspended fraction, the fraction containing the free lysosomal enzymes, was observed. Cathepsin D specific activity increased significantly over 14 days ageing in all fractions with the specific activity in the suspended fraction increasing 2 fold from 0 to 14 days. This was most probably due to the *in situ* maturation mechanism for cathepsin resulting in their activation (Chambers *et al.*, 1994).

Several factors influence the activity of the lysosomal enzymes once released from the lysosomes (Moeller *et al.*, 1976; Etherington, 1984; Barrett, 1987; Bond and Butler, 1987). The cathepsin activity is enhanced in electrically stimulated carcasses due to the early release of the cathepsins from the lysosomes as a result of the rapid

pH decline while the temperature is still high (Etherington, 1984). Cathepsin activity is controlled by a number of protease inhibitors, referred to as cystatins, located in the cytoplasm (Barrett, 1987; Bond and Butler, 1987). Cathepsin activity has also been reported to be influenced by high temperature conditioning immediately post slaughter. Moeller *et al.* (1976) reported that beef sides chilled at 22°C for 4 hours post slaughter followed by temperature conditioning at 12°C for an additional 8 hours before chilling at 2°C had a higher cathepsin activity in the muscle. This was thought to be due to the high temperature enhancing the disruption of lysosomal membranes.

Contrary results have been obtained regarding the involvement of cathepsins in tenderisation. Zeece *et al.* (1986) investigated the proteolytic activity of cathepsin D in ovine muscle and found that both increasing pH and lowering the incubation temperature to 15°C substantially depressed the cathepsin D proteolytic activity while reducing incubation temperature to 5°C caused activity to stop. Thus they concluded that this enzyme was not involved in the tenderisation of postmortem muscle as it was not active at pH and temperatures typically found in meat. Other studies by various researchers on pork, chicken, lamb and beef muscle have also indicated that lysosomal enzymes did not contribute to postmortem tenderisation (Etherington *et al.*, 1987; Furuno and Goldberg, 1986; Goodman, 1987; Koohmaraie *et al.*, 1988a and b; Lowell *et al.*, 1986). In contrast, several researchers have shown evidence of the involvement of cathepsins in tenderisation. Jiang *et al.* (1992a) has reported that the disappearance of myofibril z-discs and proteolysis of myofibrillar proteins occurred during incubation with cathepsins. Jiang *et al.* (1990) demonstrated that proteolysis of z discs by pepstatin sensitive proteases occurs in myofibrils postmortem. The proteolysis resulted in the disappearance of α -actinin on SDS-PAGE profiles of myofibrils incubated with crude proteases at pH 5.5 and 6.0. Variations in assay procedures such as different substrate concentrations or lack of inhibitor removal prior to assay may have contributed to conflicting results between research groups. The question arises, does the pepstatin-sensitive proteases have to be maximally active to cause sufficient damage to the myofibrils to produce the observed postmortem tenderness?

Cathepsin D is believed to be a highly stable enzyme and the most important cathepsin in endogenous muscle protein degradation with the other cathepsins contributing after the initial breakdown by cathepsin D (Jiang *et al.*, 1990). Jiang *et al.*

(1990) hypothesised that cathepsin D initiates the protein hydrolysis and produces peptide fragments that can then be further broken down by the other cathepsins. Toldra and Etherington (1988) have shown cathepsin D to be extremely stable with no decrease in activity during pork storage at 4°C over 20 days. Studies by Jiang *et al.* (1992) have suggested the other lysosomal proteases play a role in tenderisation by enhancing the proteolytic activity of cathepsin D. Huang and Tappel (1971) and Makinodan and Ikeda (1976) have reported cooperative proteolysis activity of cathepsins C and D and cathepsins A and D on muscle proteins. Caldwell and Grosjean (1971) suggested that cathepsins A, B and C could hydrolyse the primary reaction products produced by cathepsin D and consequently increase cathepsin D activity. It is hypothesised that cathepsin D would initiate the hydrolysis of myofibrils and propagate the hydrolysis after the removal of the primary products, during the initiation reactions by the lysosomal peptidases, thus elevating the activity of cathepsin D (Jiang *et al.*, 1992a).

Cathepsins have also been shown to continue protein degradation following the initial breakdown by calpains. Studies by Calkins and Seidman (1988) suggest that calpains help to establish initial (day one) meat tenderness but the cathepsins B and H are responsible for the tenderisation that occurs during ageing in beef. This study indicated that collectively cathepsins B and H accounted for 35% of the variation in shear force change between day one to day 14 and 58% of the variation between day three to day six (Calkins and Seidman, 1988). μ -Calpain has been demonstrated to have a reduction in activity of about 50% by day one post slaughter (Koohmaraie *et al.*, 1987) while the cathepsins would be anticipated to continue proteolysis since an acidic pH closer to their optima would be obtained by day one post slaughter. Furthermore, elevated activities of cathepsins may be partly responsible for lowering shear forces during the ageing process with cathepsin B activity being significantly related to the overall change in shear force (Koohmaraie *et al.*, 1987).

1.5.3 Tenderness measurements:

Numerous methods have been established to assess meat tenderness (Bouton *et al.*, 1975). As tenderness is probably the most important attribute of meat quality, reliable and inexpensive methods of assessment are required. Methods for assessing tenderness can be either subjective (for example, sensory panel), objective (for

example, Warner-Bratzler peak shear force) or biochemical (for example, myofibrillar fragmentation index).

1.5.3.1 *Subjective measurements:*

The main subjective method used to assess tenderness along with other eating quality characteristics is sensory panel assessment (Bouton *et al.*, 1975). Panel assessment is performed by either a trained panel or a consumer, untrained panel. The results rely on the panels physical senses to assess tenderness along with other meat quality characteristics such as juiciness, colour and flavour. Varying methods of scaling, scoring and ranking of food products are used (Bouton *et al.*, 1975).

Correlations of objective methods such as Warner-Bratzler peak shear force with subjective assessments have been reported to be highly variable (Bouton *et al.*, 1975; Bouton and Harris, 1972; Paul *et al.*, 1973; Cross *et al.*, 1973). High correlations could be obtained in circumstances where changes in the connective tissue contribution were either small or similar (Bouton *et al.*, 1975). However if differences in the connective tissue contribution were high and contrary to the Warner-Bratzler results then low correlations between Warner-Bratzler and taste panel are probable (Bouton *et al.*, 1975).

1.5.3.2 *Objective measurements:*

Due to the expense and time required to conduct taste panels subjective tenderness testing, objective measures have been developed to replace sensory testing. Tenderness is often measured mechanically with the most commonly used instruments being the Instron universal testing machine (Bouton *et al.*, 1971), the MIRINZ tenderometer (MacFarlane and Marer, 1966), and the Kramer shear press (Kramer *et al.*, 1951). The MIRINZ tenderometer and Warner-Bratzler shear device fitted to the Instron universal testing machine assess tenderness by shearing through meat samples perpendicular to the fibres. For the compression method the force required and the work done to drive a plunger through a meat sample provides an index of cohesiveness or ease of breakdown of the material. The adhesion test involves a tensile measurement in which the force required to pull a sample apart is recorded (Bouton *et al.*, 1975).

Differences for the values obtained exist between the different methods used to assess tenderness. Studies by Bouton and Harris (1972) indicate that compression measurements are more strongly influenced by connective tissue than Warner-Bratzler peak shear force measurements. The studies by Bouton and Harris (1972) found that high connective tissue of the *semimembranous* and *semitendinous* resulted in higher compression values than *longissimus dorsi* and *gluteus medius* which have lower connective tissue contents. On the other hand, Warner-Bratzler peak shear force measurements are better correlated with myofibrillar or muscle fibre properties than with connective tissue properties. Finally, measurements of adhesion between meat fibres gives an index of connective tissue strength (Bouton *et al.*, 1975).

Careful removal, handling and processing of samples is required before assessment for Warner-Bratzler peak shear force. Many factors have been shown to significantly affect the ultimate results including the method of cooking, temperature of sample cores when sheared, sample core size and sample core location (Hedrick *et al.*, 1968). Furthermore Warner-Bratzler peak shear force values have been shown to be significantly reduced if they have been frozen and allowed to thaw prior to cooking (Ferrier and Hopkins, 1997). In beef a Warner-Bratzler peak shear force value of 4.5 kg or less has been suggested to ensure high levels (98%) of consumer acceptability (Huffman *et al.*, 1996).

1.5.3.3 Biochemical methods:

Both myofibril fragmentation index and SDS-PAGE are biochemical methods to assess meat tenderness. Myofibril fragmentation index (MFI) has been developed as a biochemical method of assessing tenderness (Culler *et al.*, 1978). This method has been shown to account for more than 50% of the variation in loin steak tenderness in beef and myofibrillar fragmentation is believed to be more important in its prediction of tenderness in loin steaks than collagen solubility and sarcomere length (Culler *et al.*, 1978). A loin steak with a MFI of more than 60 should be very tender while a steak with a MFI of about 50 should be slightly tender. An MFI of less than 50 would denote a lack of tenderness (Culler *et al.*, 1978).

MFI measurement is based upon the absorbance of light by a myofibril suspension (Olson *et al.*, 1976). The absorbance method is based on the premise that at a constant myofibril concentration the amount of light absorbed by a myofibril suspension will be proportional to the relative size of myofibrils and fibre pieces in the suspension. A suspension having more fragmented myofibrils and thus smaller pieces will be more turbid and have a higher absorbance value than a suspension with longer myofibrils but the same concentration of protein (Olson *et al.*, 1976). Due to fragmentation of the myofibrils during ageing, increases in MFI are observed to occur with post slaughter storage.

Degradation of myofibrillar proteins during ageing can be studied using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). By separating the proteins on molecular weight, changes in the intensity of bands, the disappearance of bands and the formation of new bands can be observed. The principal degradative change during ageing detectable by SDS-PAGE of myofibrils is loss of troponin T, an essential regulatory protein located on the thin actin filament, and the simultaneous formation of a 30kDa peptide (Geesink, 1993). Loss of troponin T has been related to meat tenderness but it seems unlikely that degradation of this protein itself causes increased tenderness since it has no known role in structurally stabilising myofibrils (Ouali, 1990). The degradation of titin and nebulin has also been observed during ageing (Geesink, 1993). These proteins may make an important contribution to tenderness as their degradation is believed to be a cause of the weakening of thin filaments in the I and Z bands (Steen *et al.*, 1997). Differences in both titin and nebulin degradation have been observed with increased degradation occurring in more tender meat (Huff-Lonergan *et al.*, 1995) while no relationship between tenderness and titin degradation has been reported by others (Fritz *et al.*, 1993).

The complexity of the protein patterns observed in SDS-PAGE makes it difficult to demonstrate the disappearance of a minor protein or to identify the source of peptide fragments. Thus immunoblots, in particular Western Blot analysis can be used to identify changes to specific proteins due to post slaughter storage. The application of specific antibodies to the Western Blots enable a more definite identification of specific protein bands than Coomassie stained gels (Fritz and Greaser, 1991). Furthermore, the polyclonal antiserum used enables the identification of the intact

peptide as well as any fragments of that protein that still retain antigenic sites (Bandman and Zdaris, 1988).

1.6 FACTORS INFLUENCING PORK TENDERNESS

Many factors have been shown to influence pork tenderness and rate of tenderisation. These factors include animal factors, stunning method, chilling method, electrical stimulation, accelerated processing, cold shortening, pH decline and temperature decline, osmotic pressure and ion infusion. These factors will be discussed in greater detail in the following review.

1.6.1 Genetic factors

1.6.1.1 Genetics:

With the demand for leaner pork and pork products by the consumer, changes in genetics have been required and this has had a significant impact on pork eating quality. With the reduction in fatness of pig carcasses, a corresponding reduction in meat tenderness and an increased muscle lightness has been observed (Petersen *et al.*, 1997) along with a higher incidence of PSE (Grandin, 1996). The physical properties of meat quality have been shown to be moderately inheritable for both pigs and cattle (Hermesch, 1997; Judge *et al.*, 1989). Furthermore, glycolytic rate post slaughter, muscle colour, firmness, intramuscular fat and tenderness can all be influenced by the selection of breeding animals.

An increase in meat toughness has been associated with a reduction in beef carcass fatness due to alterations in the rate of muscle temperature decline post slaughter (Marsh *et al.*, 1980-81; Purchas and Davies, 1974). This increased toughness due to a reduction in fat may be attributed to a faster temperature decline which could be inducing cold shortening. Cold shortening has been reported to be lower in pork relative to beef (Moller and Jensen, 1993) due to the faster rate of glycolysis of pigs and the thicker subcutaneous fat depth providing thermal insulation. With a reduction in subcutaneous fat due to genetic changes, the extent of cold shortening may increase. Studies in beef have shown that tenderness differences between fat and lean carcasses are related to the carcass temperature differences between the 2 groups in the very early post slaughter period (Marsh *et al.*, 1980-81). Furthermore,

Marsh *et al.* (1980-81) showed that the tenderness of a lean carcass is increased significantly if the side is cooled at a rate resembling that of the fat side for 2-3 hours after dressing.

The eating quality of pork may be influenced by the level of intramuscular fat, a carcass characteristic that is highly heritable (Wood, 1993). The intramuscular fat, consisting of the lipid formed in the perimysial connective tissue surrounding the muscle fibre bundles (Wood, 1993) may influence eating quality by reducing the force required to shear myofibrils and aids the separation of fibre bundles during eating (Wood, 1991). However there is disagreements on the importance of these effects (Wood, 1991). Intramuscular fat constitutes 0.5 to 2.5% of muscle wet weight in the longissimus muscle varying with breed (Wood, 1993). The level of intramuscular fat appears to be breed related since the white European breeds contain lower levels of intramuscular fat than the dark skinned breeds such as the Duroc. Furthermore, it has been demonstrated that by increasing the percentage of Duroc genes from 0 to 50% results in improvements in tenderness by 0.4 scale points on an 1-8 scale, a detectable improvement by consumers (Wood, 1993).

1.6.1.2 *Halothane Gene*

The halothane gene or *ryr1* gene has important detrimental effects on meat quality especially the incidence of PSE pork (Wood, 1993) but produces a desirable carcass composition. Pigs carrying the halothane gene have been selected for their high lean content and thick muscles. The halothane gene is an inherent mutation in pig populations which results in the musculature being hypersensitive to stimulation by stressors. Pigs carrying the halothane gene were originally detected by exposure to halothane which resulted in a stress response in the halothane positive and heterozygote pigs. Pigs with the halothane gene show alterations in calcium movement from the sarcoplasmic reticulum, elevating the calcium concentration in the sarcoplasm due to increased release of calcium. This is due to alterations in the amino acid sequence of the sarcoplasmic reticulum Ca^{2+} - release channel membrane protein (Hermesch, 1997). These changes in calcium movement cause the prolonged activation of muscle contraction and increased glycolysis post slaughter (Greaser *et al.*, 1969) and are associated with the increased PSE observed in halothane gene positive pigs due to rapid pH fall.

Pigs which are homozygous positive (nn) and heterozygous (Nn) pigs for the halothane gene are observed to have a faster rate of pH decline post slaughter resulting in more PSE, a higher level of drip loss and a lighter surface colour (Channon *et al.*, 1997; Wood, 1993). This accelerated rate of glycolysis has been attributed to the greater stress susceptibility of these pigs to any changes in their environment. Also, a reduction in tenderness has been observed in pigs containing the halothane gene (Sather *et al.*, 1991; Bejerholm, 1984) due to the PSE conditions that result. Studies by Channon *et al.* (1997) reported no differences in tenderness between heterozygous pigs (Nn) and normal (NN) pigs at 1 day post slaughter but by 5 days post slaughter the carrier pigs had a reduction in tenderness compared to the normal pigs. The increased toughness of the carrier pigs after 5 days ageing probably reflects the increased incidence of PSE and the reduction in ageing associated with PSE muscles (Warner, 1994; Fernandez and Tornberg, 1994).

1.6.2 Processing factors

1.6.2.1 Stunning method:

Pigs are stunned prior to slaughter in order to make them insensible to pain prior to exsanguination. There are two main forms of stunning systems used in the Australian and overseas pig industries - carbon dioxide stunning and electrical stunning. In recent years the use of carbon dioxide stunning in Australia has increased due to improved worker safety, reduced incidence of ecchymosis, reduction in PSE and a reduction in bone fractures (Channon, 1996; Barton-Gade, 1993; Barton-Gade *et al.*, 1990).

Carbon dioxide stunning involves immersing the animal into a carbon dioxide atmosphere which consists of 85-90% carbon dioxide in air, for 1-2 minutes, which results in anaesthesia of the pigs. After the initial exposure to carbon dioxide, the pigs remain calm for approximately 10-15 seconds prior to uncontrolled muscle activity lasting 5 to 7 seconds. After 30 seconds of exposure to carbon dioxide, a deep anaesthesia is reached and the animals are relaxed (Channon, 1996). Exsanguination within 10 seconds of stunning is required to achieve death before

sensibility is regained and to ensure that the animals remain perfectly still (Barton-Gade *et al.*, 1990).

Electrical stunning involves the use of electricity to produce an epileptic fit to render the animal unconscious and insensitive to pain. The electric current can be applied using tongs on the head only, on the head and chest, or on the head and back. When head to chest tongs are used, a cardiac arrest is induced thereby killing the animal. Electrical stunning induces unconsciousness by causing a disorganisation of the normal brain activity. The physical response following stunning can be divided into two phases. The initial phase lasts about 10 to 20 seconds resulting in the raising of the head and flexion of the legs towards the body. The second phase lasts from 15 to 45 seconds and is characterised by excessive kicking making exsanguination dangerous (Gregory, 1985). Although the heart action stops following head to chest stunning this does not negatively influence bleed out (Channon, 1996).

Muscular contractions during and after electrical stunning has been seen to have a negative effect on pork quality in particular colour and water holding capacity. Van der Wal *et al.* (1997) reported that high levels of muscular contraction caused a more rapid drop in pH, a faster development of rigor onset and a reduced water holding capacity. Furthermore, the level of muscular contractions were seen to increase with imperfect electrical stunning procedure, increasing the negative impact on pork quality.

The majority of research into stunning has concentrated on animal welfare and worker safety with little research investigating overall eating quality. Carbon dioxide stunning reduces the incidence of PSE (Channon *et al.*, 1997; Barton-Gade, 1993). This reduction in PSE is due to lower levels of catecholamine secretions during stunning with carbon dioxide (Larsen, 1982), less muscular activity and slower pH fall (Channon *et al.*, 1997). Furthermore, the incidence of blood splash (Larsen, 1982) and broken bones as a result of violent muscle spasms is reduced with carbon dioxide stunning (Channon *et al.*, 1997; Barton-Gade, 1993; Barton-Gade *et al.*, 1990). Further research is required to determine the impact that stunning method has on tenderness, juiciness and overall eating quality.

The method of stunning can alter the rate of pH decline and therefore ultimate meat quality. Electrical stunning increases the rate of pH decline, relative to carbon dioxide stunning (Channon *et al.*, 1997) and electrical stunning causes similar glycogen metabolism rates to that with electrical stimulation (Barton-Gade, 1993). The differences in pH decline seen in pig *longissimus* muscle after carbon dioxide stunning compared to head to heart electrical stunning seen by Channon *et al.* (1997) also corresponded to a difference in tenderness at 1 day post slaughter with the electrically stunned pigs being more tender. A higher incidence of PSE pork has also been reported in electrically stunned pigs relative to those stunned in carbon dioxide (Channon *et al.*, 1997; Barton-Gade, 1993).

1.6.2.2 *Electrical Stimulation*

Electrical stimulation of animal carcasses within 60 minutes of slaughter has been shown to improve several aspects of meat quality (Polidori *et al.*, 1996). Electrical stimulation significantly reduces cold shortening, improves tenderness, meat colour, increases marbling score (visually assessed), prevents heat ring and enhances flavour in beef and lamb carcasses (Polidori *et al.*, 1996). The main tenderising effects of electrical stimulation are (i) the prevention of cold shortening by reducing the time to rigor. This is achieved by reducing the ATP concentration and other high energy phosphates and by the rapid fall in pH due to enhanced glycolysis; (ii) increased activity and earlier activation of acid proteases due to the rapid acidification induced by electrical stimulation and (iii) physical disruption in the muscles as a direct result of the application of electricity resulting in the absence or poorly defined A, I and Z bands (Sorinmade *et al.*, 1982).

The magnitude of the effects of electrical stimulation on meat quality for all species varies with the type and method of electrical stimulation. Two main forms of electrical stimulation are employed - low voltage electrical stimulation and high voltage electrical stimulation with 'extra' low voltage electrical stimulation being employed in Australia due to occupational health and safety regulations on voltage. Low voltage electrical stimulation is defined as a voltage of 20-90V, < 1 amp and must be applied within 10 minutes post slaughter to be effective as the stimulation of the nervous system by the current relies on the intact nervous system (Savell, 1985). High voltage electrical stimulation usually consists of a voltage of 550-600V and 5-15A and

can be used at any time within 60 minutes of stunning as the current is spread by direct membrane depolarisation (Marton and Newbold, 1982).

Electrical stimulation of a carcass results in the acceleration of rigor development (Aalhus *et al.*, 1994). This accelerated rigor development is due to extensive membrane depolarisation and muscular contractions during the application of the stimulation (Aalhus *et al.*, 1994). The membrane depolarisation and muscular contraction initiates a rapid depletion of ATP and glycogen resulting in an increase in lactate and fall in pH (Aalhus *et al.*, 1994). Due to the rapid fall in pH while the muscle temperature is still high the possibility of cold shortening is reduced (Polidori *et al.*, 1996). However, care must be taken when electrical stimulation is applied to pigs to ensure the extent of pH fall is not great enough to induce PSE.

The fall in pH due to stimulation occurs in 2 stages (Chrystall and Devine, 1992). The initial stage involves a fall during the stimulation period at a rate that is 100-150 times that of the normal rate. This initial stage is believed to continue until either stimulation is stopped or a pH of about 6.2 is attained, whichever occurs first (Takahashi *et al.*, 1984). The magnitude of the initial phase is highly dependent on the time of stimulation with a greater impact occurring with stimulation within 5 minutes post slaughter relative to stimulation at later stages (Polidori *et al.*, 1996). The time that electrical stimulation is applied after exsanguination is an important factor influencing pH decline and subsequently tenderness (Chrystall and Devine, 1985). If stimulation is delayed, muscle temperature may have fallen which reduces the potential magnitude of the change in pH (Chrystall and Devine, 1978). Furthermore, glycolysis will have progressed further reducing the potential magnitude of the change in pH. Finally, delaying low voltage stimulation can reduce its effectiveness due to the decay of the nervous system, causing the carcass to become unresponsive to the stimulation (Devine *et al.*, 1979). Delays in stimulation are less effective in reducing pH due to the possibility of a fall in muscle temperature, progression of glycolysis and the decay of the nervous system, all reducing the magnitude of pH change (Chrystall and Devine, 1992). The second stage is the fall in pH following the cessation of the stimulation and is 1.5-2 times higher than the normal rate of pH fall (Chrystall and Devine, 1992). It is the magnitude of the initial pH fall that seems to determine whether muscles lose their capacity to cold shorten (Chrystall and Devine, 1985) as

this ensures that the pH is less than 6 before the muscle temperature falls below 10°C, conditions which are required to prevent cold shortening.

Different wave forms can be employed during electrical stimulation of carcasses (Chrystall and Devine, 1992). Waveforms can be unipolar or bipolar and are applied as discrete pulses or as pulse trains (Chrystall and Devine, 1992). Furthermore, the frequency of the applied voltage may be altered with frequency optimums of around 9-16 pulses/second for sheep and beef carcasses (Polidori *et al.*, 1996).

The biochemical events that occur as a result of electrical stimulation are similar to those that occur when pre-rigor muscle is conditioned at elevated temperatures (Dutson and Pearson, 1985). Both electrical stimulation and temperature conditioning result in a lowering of the pH while temperatures are still high thereby preventing cold shortening. In addition, there is a minimisation of reduced calpain autolysis which occurs in cold conditions and both electrical stimulation and temperature conditioning increase the free activity of lysosomal enzymes (Dutson and Pearson, 1985).

Savell (1979) reported that the muscles from electrically stimulated carcasses require less ageing than unstimulated carcasses in order to reach an acceptable level of tenderness. They found that ageing of meat from stimulated beef carcasses for 14 days resulted in 15% improvement in tenderness whereas non-stimulated carcasses had a 26% improvement. However the electrically stimulated loins were 10% more tender after 21 days ageing than non-stimulated loins. This indicates that ageing periods can be reduced after the application of electrical stimulation.

The effectiveness of electrical stimulation in improving tenderness depends upon the chilling method post slaughter (Takahashi *et al.*, 1984; Chrystall and Devine, 1985; Unruh *et al.*, 1986). Electrical stimulation is most effective at improving tenderness when carcass cooling is fast enough to induce cold shortening due to the prevention of sarcomere length shortening and the earlier activation of the tenderising enzymes. The effects of electrical stimulation on meat tenderness can also be seen but to a lesser extent when chilling is still rapid but not rapid enough to induce cold shortening. However, toughening has been seen in electrically stimulated muscles where a very slow rate of cooling has been used (Takahashi *et al.*, 1984; Unruh *et al.*,

1986) or when pre-slaughter stress has occurred, which results in accelerated glycolysis to such an extent that rigor develops close to or during stimulation resulting in heat toughening (Chrystall and Devine, 1985) and PSE conditions.

Tenderness has been reported to be improved following stimulation even when cold shortening does not occur. Dutson *et al.* (1980) concluded that electrical stimulation generally improved tenderness even though no differences in sarcomere length may be evident between the non stimulated and stimulated muscles. The improved tenderness is believed to be due to electrical stimulation causing a rapid release of lysosomal enzymes and/or by the physical disruption of the electrically stimulated muscle fibres (Dutson *et al.*, 1977) and due to the earlier activation of the tenderising enzymes. Electrical stimulation has been shown to cause mechanical damage as a result of muscular contraction while the stimulation is being applied (Sorinmade *et al.*, 1982). Electrically stimulated muscles have been shown to exhibit irregular bands, stretched myofibrils and empty spaces resulting from missing or absent myofibrils, all of which contribute to the increased tenderness associated with electrically stimulated muscles (Sorinmade *et al.*, 1982).

The effects of electrical stimulation depend upon the muscle fibre type with fast twitch muscles responding differently than slow twitch muscles (Devine *et al.*, 1984). After stimulation, fast twitch muscles such as the *cutaneous trunci* show a significant drop in pH and an increase in the rate of pH fall. In contrast slow twitch muscles such as the *masseter* have a small decrease in pH with no increase in rate of pH fall (Devine *et al.*, 1984). Muscles made up of both fast and slow twitch muscle fibres such as the *longissimus dorsi* are reported to show intermediate responses to electrical stimulation with a moderate change in pH and an intermediate change in the rate of pH fall (Devine *et al.*, 1984).

1.6.2.3 Accelerated processing

Two major processing methods are employed throughout the world, conventional and accelerated processing. Traditionally, most abattoirs bone and process pig carcasses post rigor, chilling the carcasses to a deep butt temperature of 10°C over a 14-16 hour chilling period (James, 1987) which is 'conventional processing'. This process requires large chill rooms that are often overloaded, contributing to meat

quality problems such as excessive drip loss during subsequent cutting as a result of slow initial chilling (James, 1987).

An alternative to this traditional chilling method is the boning and processing of carcasses pre-rigor, since this has many economic advantages over conventional processing (van Laack, 1989). The processing of carcasses prerigor is known as hot boning or accelerated processing. Accelerated processing implies boning the carcass before the body temperature has been substantially reduced by chilling (van Laack, 1989). Pork processed this way has been reported to reduce cooler/storage space, reduce energy input, increase product turnover, improve sanitation and shelf life, reduce drip in vacuum bags, reduce labour, material and equipment costs, and improve processing properties of the meat (Cross and Siedman, 1985; Powell *et al.*, 1982). It has been estimated that accelerated processing of beef carcasses could require 40-50% less refrigeration input than conventionally processed beef, a 50-55% reduction in cooler space, 25% reduction in labour and a 2% reduction in shrinkage (Kastner, 1982).

Accelerated processing has been reported to increase meat yield and reduce evaporative losses (Visser *et al.*, 1976). These evaporative losses can be accounted for in conventionally processed muscle by the difference in water vapour pressure between the surrounding air and the carcass surface, evaporative losses induced by force air circulation around the carcasses and evaporative losses due to heat transmission from the carcass surface (Visser *et al.*, 1976). By placing primals from accelerated processed carcasses into vacuum packaging in moisture proof film before chilling, much of this evaporative loss can be prevented.

Improvements in colour and colour uniformity have been reported to occur in accelerated processed beef (Taylor *et al.*, 1981). The improvement in colour has been attributed to the greater surface area exposed to cold temperature during chilling and the even cooling process along with a decrease in protein denaturation.

Although economic benefits and a high quality product can result from accelerated processing, widespread commercial application has not been realised. The major reasons that the implementation of accelerated processing has been limited are due to the following factors; rapid chilling of hot meat tends to produce tougher meat due

to cold shortening, greater temperature and hygiene control requirements, unconventional shapes of cuts tend to occur, the difficulty in incorporating hot boning into conventional plants and no system is developed for rapid chilling of large volumes of cuts (Cross and Seidman, 1985; Powell *et al.*, 1982).

While fast chilling of conventionally processed carcasses has some benefits, chilling of accelerated processed muscles must be carefully monitored. Chilling rates after accelerated processing must be controlled to avoid cold shortening of the muscle which results in tougher meat (see 1.7.3.1). Furthermore, accelerated processing affects the rate of pH/temperature decline during the onset of rigor mortis and thus enzymatic processes which may directly or indirectly affect the colour stability of meat (van Laack, 1989).

1.6.2.4 Chilling

The rate of chilling post slaughter can influence the ultimate tenderness of pork due to effects on glycolytic rate. The rate of chilling of muscles post slaughter is influenced by many variables. These variables include the size, shape and fatness of the carcass and the temperature, relative humidity and flow pattern of the chiller air. Carcass characteristics are thought to be at least as important in determining muscle cooling rate as the temperature and velocity of the chiller air (Lochner *et al.*, 1980).

Muscle pH early post slaughter is closely linked to muscle temperature through its influence on the rate of glycolysis and thus the rate of pH decline. When temperatures are above values associated with cold shortening (10°C), a positive relationship between temperature early post slaughter and tenderness has been found (Marsh *et al.*, 1981; Lochner *et al.*, 1980). Differences in the chiller temperature at which carcasses are initially stored can bring about distinct changes in the rate of chemical reactions. A difference of 10°C in the chilling temperature can result in a change in rate of glycolytic reactions by a factor of three or more (Judge *et al.*, 1989). Thus it is important to quickly reduce carcass temperature to less than 18°C by 6-8 hours post slaughter to prevent protein denaturation (Trout, 1993) and to inhibit microbial growth without causing cold shortening. Temperature decline post slaughter also plays an important role in determining tenderness.

The duration of chilling time for pig carcasses impacts on the economic efficiency of the pig industry. Reducing the chilling time would be beneficial in reducing evaporative loss, drip loss and the growth of spoilage organisms. Rapid chilling has been recommended as a way of reducing quality problems associated with PSE pork. On the other hand, cold toughening caused by rapid chilling, particularly in slow glycolysing muscles is a risk (Iversen *et al.*, 1995).

The use of blast chilling for pork is increasing. Blast chilling results in a rapid fall in carcass temperature early post slaughter due to initial chilling at -30 to -20°C for several hours before chilling at 0 to 2°C (Feldhusen and Kuhne, 1992). The increased use of blast chilling is due to its ability to shorten chilling time before fabrication. Blast chilling reduces the occurrence of PSE by reducing muscle temperature and slowing glycolytic rates (Borchert and Briskey, 1964) and extends the shelf life of pork (McFarlane and Unruh, 1996). However care must be taken to prevent cold shortening and disruption of the ultrastructure of the muscle as a result of freezing surface muscles which can result in a reduction in water holding capacity (Honikel *et al.*, 1986; Londahl and Eek, 1986).

1.6.2.5 Calcium chloride infusion:

Tenderisation of beef and lamb has been shown to be improved by the injection of calcium chloride into pre-rigor muscle. Several studies have shown that injection of 0.3M CaCl₂ into pre-rigor bovine muscle has resulted in almost complete tenderisation within one day post slaughter and CaCl₂ treated muscles are more tender than non treated muscle even after complete ageing where no improvements in tenderness are observed with further storage (Morgan *et al.*, 1991; Wheeler *et al.*, 1991; Geesink, 1993). Studies by Boleman *et al.* (1995) showed that injection of beef semimembranosus at one hour post slaughter was effective in reducing shear force values and preventing excess moisture loss. They also showed that injection at 12 or 24 hours post slaughter was effective in lowering shear force values.

There are several proposed mechanisms for the tenderness improvements after calcium chloride infusion (Koohmaraie *et al.*, 1989; Morgan *et al.*, 1991; Wu and Smith, 1987). Koohmaraie *et al.* (1989) reports that the tenderising effect of calcium chloride is due to the activation of the calcium dependent proteases *u*- and *m*-calpain

accelerating the proteolysis of myofibrillar proteins. Morgan *et al.* (1991) proposes that injection results in extreme contraction of the muscle fibres which may result in the disruption of the myofibrillar network thus improving tenderness. Wu and Smith (1987) propose that the increase in tenderness may be due to the elevated ionic strength influencing the protein to protein interactions and protein solubility (see section 1.6.4.2). The aggregation of proteins into myofibrils involves electrostatic interactions which are influenced by ionic strength. By increasing the ionic strength the concentration of monovalent ions is raised, the sphere of each charge on the proteins is decreased and this may cause a weakening in the structural integrity of the myofibrils. Divalent ions, due to their high affinity for opposite charges, can act by blocking the electrostatic interactions between charged groups of the proteins, again causing a weakening in the structural integrity of myofibrils. Thus elevated ionic strength has little effect on the z-lines but causes solubilization of proteins from thick and thin myofilaments (Wu and Smith, 1987).

Improvements in tenderness have also been seen with the infusion of muscle with other ions. Studies by Alarcon-Rojo and Dransfield (1995) showed that sodium and potassium chlorides were 43% and magnesium chloride 73% as effective as calcium salts in tenderising beef when *M. semitendinosus* were soaked in a solution at 10°C 24 hours post slaughter. In contrast 1 mM EDTA, a strong calcium chelator, had no positive or negative effect on tenderising. These results suggest that addition of ions has a large ionic effect on tenderness.

Calcium chloride has been found to improve meat tenderness in muscles that are extremely tough. Feeding some β -adrenergic agonists to sheep improves feed efficiency and carcass composition but a significant reduction in meat tenderness may result which calcium chloride can overcome (Ertbjerg *et al.*, 1994). Ertbjerg *et al.* (1994) demonstrated that in lamb calcium chloride has no effect on cathepsin B, and B+L activities but has a significant effect on μ -calpain, *m*-calpain and calpastatin activities therefore the tenderising effect was thought to be due to the activation of the calpain enzymes and not due to alterations in ionic strength.

1.6.3 Mechanical factors

1.6.3.1 Cold shortening

Cold shortening also referred to as cold toughening is the reduction in sarcomere length of muscle fibres due to exposure to cold temperatures (Dutson and Pearson, 1985). Cold toughening is believed to be a better expression as it incorporates the increased toughness due to both a reduction in sarcomere length and a reduction in proteolysis due to the cold temperature (Dutson and Pearson, 1985). Although cold shortening is greater in beef and lamb than in pork (Kauffman and Marsh, 1987), cold shortening does occur in pork when chilling conditions are severe (Moller and Jensen, 1993).

The relationship between cold shortening and tenderness is complex (Marsh and Leet, 1966). A decrease in sarcomere length in beef of 20% from initial length does not appear to effect tenderness when assessed at 2 days post slaughter but tenderness decreases rapidly with further shortening reaching a peak with shortening of approximately 40%. With further shortening, tenderness once again improves (Marsh and Leet, 1966). Due to the long ageing period of beef, these results are unlikely to be influenced by ageing. In contrast to this Locker (1985) and Lee (1984) reported that a loss in tenderness has been shown to occur with sarcomere shortening of 20%. Cold induced muscle shortening in pre-rigor excised *longissimus dorsi* muscle from pork has been reported to be 34% (Dransfield and Lockyer, 1985) while for beef up to 50% shortening has been observed at chilling temperatures of 2°C (Chrystall and Devine, 1985).

Cold shortening results from a rapid fall in muscle temperature early post slaughter while muscle pH is still high, initiating calcium release from the sarcoplasmic reticulum and the mitochondria (Pearson and Young, 1989). This fall in temperature causes changes in the ability of the sarcoplasmic reticulum and mitochondrial to reabsorb calcium resulting in an increase in calcium ion concentration in the myofibrillar space (Kanda *et al.*, 1977a,b; Cornforth *et al.*, 1980). This raised calcium concentration initiates muscle contraction before the onset of rigor mortis. As the calcium ions are not reabsorbed by the sarcoplasmic reticulum, the binding of the calcium to the troponin system in the thin filaments releases the blocking effect of tropomyosin on actin, permitting the cycling interaction between actin and myosin to

continue. This causes severe contraction and a permanent reduction in the sarcomere length due to the deficiency of ATP to remove the interaction between actin and myosin, thereby increasing meat toughness (Hamm, 1981).

Cold shortened muscle samples can be considerably tough (Pearson and Young, 1989). Warner-Bratzler shear force values of 80 N/cm² have been observed in cold shortened muscle, considerably above the level of 60 N/cm² normally considered to be at the borderline of acceptable tenderness when evaluated by taste panel (Iversen *et al.*, 1995). This increased toughness following cooking is believed to be due to a greater filament density as a result of the overlapping of the filaments with a shortening of sarcomere length (Iversen *et al.*, 1995) and a reduction in proteolytic activity.

The ability to cold shorten persists while the pH is above 6.2 (Dransfield, 1994) regardless of the processing method. As most conventionally processed muscles are restrained during cooling by tendons and bones, the cold shortening is less severe than in hot boned muscles (Polidori *et al.*, 1996). Shortening can occur in muscles that are fixed at both ends. For example, the *longissimus* retained on the carcass has been reported to shorten as most of the constituent fibres insert into flexible epimysium allowing the muscle to shorten (Polidori *et al.*, 1996).

The extent of cold shortening is dependent upon several factors (Devine *et al.*, 1984; Dutson and Pearson, 1985). For example, muscles from older animals have been shown to have a greater capacity to cold shorten than those from younger animals (Dutson and Pearson, 1985). Furthermore, red muscles have been shown to be more susceptible to cold shortening than white muscles (Devine *et al.*, 1984). Red muscles have a higher concentration of mitochondria which under cold temperatures prevent the absorption of large amounts of calcium from the intracellular space. As red muscles also contain less sarcoplasmic reticulum, the muscles become overloaded with free calcium which results in more extensive muscle shortening. White muscles with fewer mitochondria do not release as much calcium thus the calcium ions are more readily recaptured by the abundant sarcoplasmic reticulum thereby minimising shortening (Pearson and Dutson, 1985). In addition, white muscles contain a higher concentration of ATP, further aiding in the prevention of cold

shortening by providing energy for the reaccumulation of calcium in the sarcoplasmic reticulum and mitochondria.

Cold shortening can be reduced by careful control of chilling or by temperature conditioning (Pearson, 1986; Honikel and Reagan, 1987; Pearson and Young, 1989). Temperature conditioning results in slower temperature decline as the muscles are chilled at higher temperatures than normal. Holding pre-rigor meat at temperatures around 16°C until the onset of rigor mortis can be used to prevent cold shortening (Pearson, 1986). Chilling at 16°C slows the release of calcium ions and increases the capacity of the sarcoplasmic reticulum to take up excess calcium, thus enabling the muscles to relax (Pearson, 1986). In pig muscle with slow or normal glycolysis and ultimate pH of approximately 5.5, onset of rigor mortis at a pH of around 6.0 must occur at 15-18°C for minimum shortening of sarcomere length resulting in low drip loss and high tenderness (Honikel and Reagan, 1987). Thus optimal conditions for normal muscle with regard to meat quality is to chill to temperatures below 20°C within four hours post slaughter and chilling not below 10°C at five hours to avoid cold shortening (Honikel and Reagan, 1987).

As an alternative to temperature conditioning, electrical stimulation has also been shown to reduce cold shortening. Electrical stimulation prevents cold shortening by causing a more rapid depletion of ATP due to an increased rate of post slaughter metabolism (Pearson and Young, 1989). The increased rate of metabolism results in a lower pH while muscle temperatures are still high and maximises proteolysis (Dutson and Pearson, 1985). However, care must be taken with electrical stimulation of pork carcasses as the rapid depletion of ATP in pork carcasses induced by electrical stimulation could result in an increased occurrence of PSE (Crenwelge *et al.*, 1984; Warriss *et al.*, 1995).

The influence of cold shortening on muscle tenderness is observed to be due to the relationship with sarcomere length (Marsh and Leet, 1966). Muscles having a sarcomere length of 2.0 to 2.5 μm have been reported to be tender, those having a sarcomere length of 1.7 to 2.0 μm moderately tender, those having a sarcomere length of 1.5 to 1.7 μm being extremely tough while those having a sarcomere length of less than 1.5 μm improving in tenderness (Marsh and Leet, 1966). These figures indicate that toughness increases as the overlapping of the thick and thin filaments

increase. With sarcomere length shortening to 1.5 μm , the thick filaments sit up against the z disc and this is referred to as the state of maximum toughness. With further shortening, the thick filament penetrates the z disc, disrupting the structure and thereby improving the tenderness (Goll *et al.*, 1997). Thus the negative impact of cold shortening on tenderness is two fold, a reduction in tenderness due to the overlapping of myofibril filaments and a reduction in tenderness due to the prevention of proteolysis.

Not only is temperature control required to prevent cold shortening, tenderness can also be influenced by temperature decline rates that do not result in cold shortening. The impact of moderate temperature decline post slaughter on tenderness was clearly seen by Herring *et al.* (1965a). In their study, bovine *semitendinosus* muscles excised pre-rigor and permitted to cold shorten were much tougher than those held in a stretched condition. Furthermore, the stretched muscles were tougher at 2 days post slaughter than the muscles removed from the carcass 2 days post slaughter although they had a shorter sarcomere length than the stretched muscles. The increased toughness of the stretched muscles can be attributed to the fact that these muscles would have had a faster temperature decline than the muscles left on the carcass until 2 days post slaughter. Thus the increased tenderness in the muscles remaining on the carcass to 2 days post slaughter can be attributed to the earlier activation of the proteolytic enzymes due to the higher muscle temperature. This suggests that improved tenderness not only results from the prevention of cold shortening but is also due to retarded decline of temperature during the first 2 to 4 hours post slaughter influencing proteolytic activity.

1.6.4 Biochemical factors

1.6.4.1 Rate of pH decline and ultimate pH

The glycolytic rate is believed to be the most important post slaughter factor influencing meat tenderness through its effects on shortening and proteolytic activity (Marsh *et al.*, 1987). Furthermore, early post slaughter temperature is believed to influence tenderness through its control over the glycolytic rate. The post slaughter rate of pH fall in combination with muscle temperature will influence muscle shortening and proteolysis. Therefore meat toughness will be influenced by the level

of shortening and the extent of proteolysis and water holding capacity will be influenced by the level of shortening. Very slow chilling rates accelerate the rate of glycolysis resulting in improved tenderness but sometimes this acceleration is so extensive that the muscle is significantly toughened (Marsh *et al.*, 1987).

Maximum tenderness has been reported with intermediate glycolytic rates (Takahashi *et al.*, 1984; Unruh *et al.*, 1986; Marsh *et al.*, 1987; Pike *et al.*, 1993). Several studies in beef have related tenderness to pH values early post slaughter. Pike *et al.* (1993) found that a quadratic relationship occurred between shear force and pH at 3 hours post slaughter. Optimum tenderness was seen with a pH at 3 hours post slaughter of 6.0 with shear force increasing as the pH was either increased or decreased from 6.0. However with ageing, shear force values declined differentially favouring the slow glycolytic, initially tougher muscles (Pike *et al.*, 1993). Other researchers have also reported this curvilinear relationship between early post slaughter pH and beef tenderness (Takahashi *et al.*, 1984; Unruh *et al.*, 1986; Marsh *et al.*, 1987) but Marsh *et al.* (1987) reported that the toughening effect of rapid glycolysis persisted throughout the 14 day ageing period. In contrast to findings of intermediate muscle pH leading to the most tender meat, Klont *et al.* (1996) reported faster glycolysing muscles to be more tender.

The results showing a curvilinear relationship between early post mortem pH and tenderness are contrary to the belief that a rapid glycolytic rate induced by electrical stimulation with a corresponding slow chilling results in optimal tenderness (Klont *et al.*, 1996, Smith, 1985). The high glycolytic rate/low temperature decline induced by electrical stimulation was believed to be optimal due to rapid glycolysis with slow chilling causes the release and/ or activation of lysosomal enzymes. Marsh *et al.* (1987) and Pike *et al.* (1993) reported that rapid glycolytic rate with slow chilling produced the greatest toughening in the absence of cold shortening. Thus cooling conditions can exert an overriding effect on tenderness through heat shortening.

The ultimate pH has also been shown to influence tenderness in beef with a quadratic relationship between ultimate pH and shear force values being observed. Studies by Purchas (1990) found that a curvilinear relationship existed between ultimate pH and shear force with shear force values increasing from pH 5.2, peaking at 6.1 before decreasing again as the ultimate pH rises up to 6.6. This could not be explained by

sarcomere length as sarcomere length was observed to decrease with an increase in pH up to approximately 6.3 and then increase slightly with increases in pH. This improvement in tenderness with increase in ultimate pH above 6.1 has been postulated to be due to changes in proteolytic activity as the calpains have a pH optimum close to 7.0.

Contrary to the curvilinear relationship seen by Purchas (1990), others have reported a linear relationship between ultimate pH and tenderness. Steen *et al.* (1997) reported that tenderness increases linearly with increase in pH over the normal range. This difference in findings has been attributed to the end point cooking temperature used for assessment with a curvilinear relationship occurring with medium cooking temperatures (65°C) and a linear increase in tenderness at high cooking temperatures (90°C) (Bouton *et al.*, 1971).

Tenderness of pork has also been observed to be influenced by the ultimate pH. Eikelenboom *et al.* (1996) reported that shear force at 3 and 7 days post slaughter and sensory tenderness could be related to the ultimate pH. An increase in ultimate pH (from 5.4 to 6.25) correlated to an increase in sensory tenderness scores and a reduction in shear force values. As the shear force at 7 days post slaughter in the study by Eikelenboom *et al.* (1996) had a better correlation with ultimate pH than at 3 days post slaughter, it was suggested that the ultimate pH had an effect on the ageing rate as well as initial tenderness.

1.6.4.2 Osmotic Pressure:

Osmotic pressure of muscle is also believed to influence meat tenderness (Ouali, 1990). Changes in osmotic pressure in post slaughter muscle may contribute to breakdown of the myofibrillar structure which is responsible for the tenderisation of meat (Ouali, 1990). Osmotic pressure can be defined as the pressure that must be exerted on the high solute concentration side of a semipermeable membrane to prevent the flow of water across the membrane due to osmosis. The aggregation of myosin molecules, and the actin-tropomyosin complex involves electrostatic interactions which in turn are influenced by ionic strength (Wu and Smith, 1987). Ionic strength has a marked influence on protein-to-protein interactions and protein solubility, thus increasing the ionic strength decreases the sphere of each charge on

the proteins resulting in a possible weakening of the structural integrity of myofibrils (Wu and Smith, 1987). Osmotic pressure has been reported to be significantly higher in post rigor than pre-rigor meat (Winger and Pope, 1980-81), thus changes in ionic strength might affect muscle properties and overall meat quality (Ouali, 1990). Wu and Smith (1987) have shown that the major effect of elevating ionic strength was to disrupt the tertiary structure of the myofibrils, suggesting that ionic strength effectively dissociates myofibrillar proteins from myofibrils.

Muscle osmolarity has been shown by Ouali *et al.* (1991) to be highly related ($r > 0.90$) to the rate and extent of pH fall, hence pH is assumed to play a major role in the observed changes in osmotic pressure. The rate of change in osmotic pressure decreases as temperature of rigor onset is raised from 10 to 30°C (Ouali *et al.*, 1991). Thus, accelerated processing may alter the ultimate osmotic pressure and therefore influence tenderness (Ouali *et al.*, 1991).

Osmotic pressure may also play a role in the water holding capacity of meat (Winger and Pope, 1980-81). It is probable that free ions contribute to the degree of swelling or shrinking of the myofibrillar filaments lattice which determines the extent of water loss in post slaughter muscle (Offer and Knight, 1988). Recent investigations performed on beef suggest that the osmotic pressure reached in post rigor meat seemed to be muscle type dependent (Ouali, 1990). Highest osmolarity is observed in fast-twitch muscles, a finding consistent with the fact that alterations during ageing of the myofibrillar structure are fastest in these muscles (Ouali, 1990).

1.7 SUMMARY:

From the literature it is clear that many factors influence the tenderness of pork and one ideal pathway can not be identified to guarantee pork tenderness. Furthermore, the effects of some of the factors on pork tenderness are difficult to interpret due to interaction with other factors. This review has highlighted some of these factors that may influence pork tenderness including genetics, stunning methods, electrical stimulation, processing method, chilling rates and post slaughter ageing.

The research in this thesis was designed to provide a greater knowledge of factors that influence pork quality, in particular tenderness, to assist in determining a

processing method that reduces processing costs and produces a consumer acceptable product. Thus a desirable pathway for the economical production of tender pork could be identified, changing the consumers perception of pork from tough and dry to tender and juicy.

Close examination of accelerated processing was undertaken to determine if pork quality was jeopardised by the process and methods to overcome these problems were investigated. The rate of ageing of accelerated processed pork was also investigated to determine if tenderness of cold shortened pork could be improved with post slaughter storage. Ageing patterns after temperature conditioning and calcium chloride infusion were examined to determine the length of time pork should be stored prior to consumption to maximise tenderness following these changes to the processing system.

To overcome the potential impact of pigs differing in genetics on experimental results, all pigs for this work were obtained from the one piggery with all pigs bred from similar genetics. The work was carried out at a pilot abattoir enabling close control of transport, handling, lairage, stunning, slaughtering and chilling. The control of genetics and the pre- and post-slaughter conditions enabled a clearer examination of treatments applied and a clearer comparison between different experiments.

Finally, the influence of low voltage electrical stimulation, stunning method and chilling rate on pH decline and rate of ageing were examined to determine the optimal slaughtering/chilling conditions for conventionally processed pork to optimise tenderness and overall pork quality. A comparison with lamb was conducted to investigate the different responses between species and between white and red muscles.

Figure 1.1. Connective tissue organisation of muscle (from Bechtel 1986).

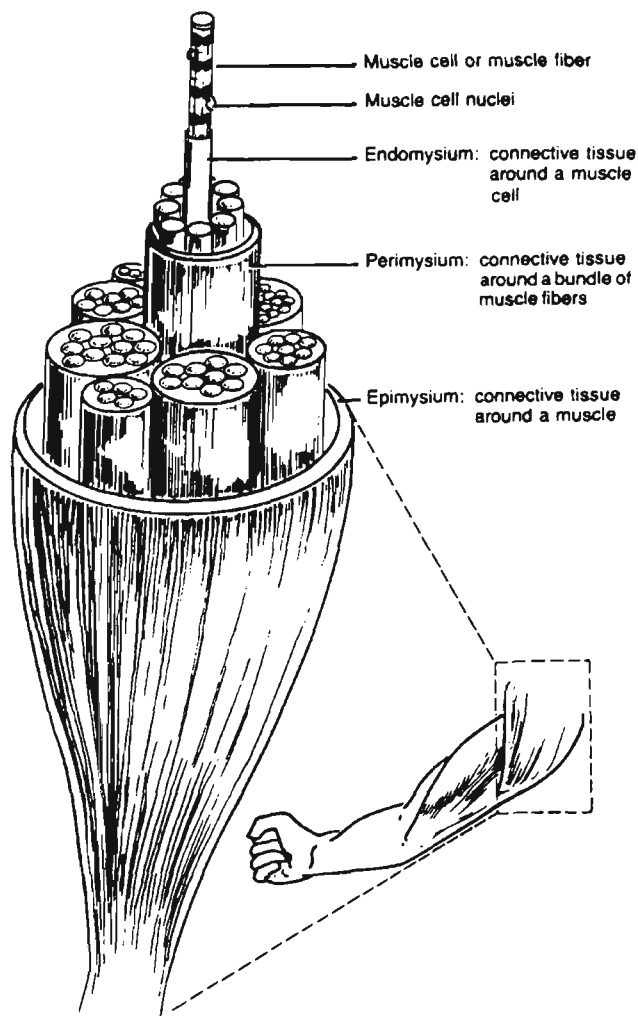


Figure 1.2. Longitudinal view of skeletal muscle (from Pearson and Young 1989)

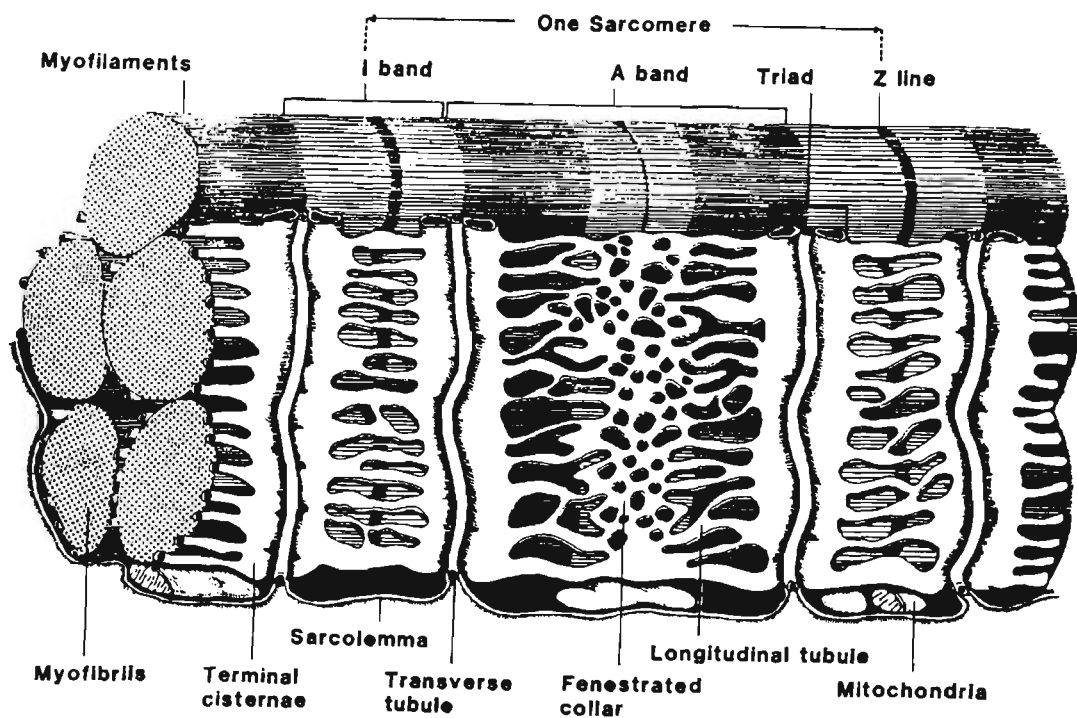
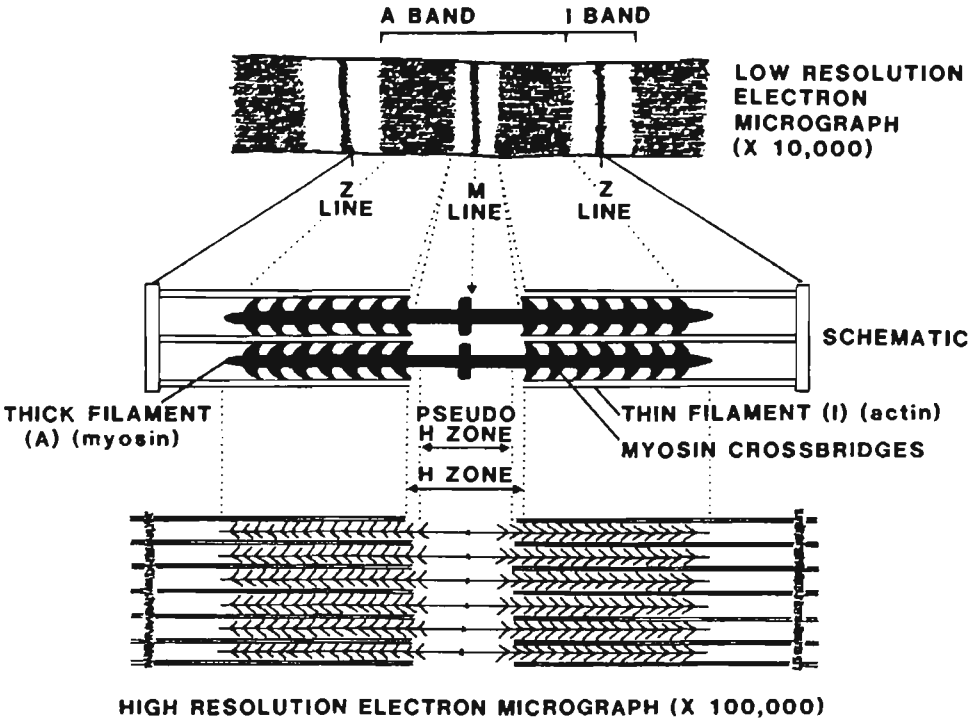


Figure 1.3. Structure of a single sarcomere (from Pearson and Young 1989)



2. MATERIALS AND METHODS

2.1 STUNNING, ELECTRICAL STIMULATION AND SLAUGHTER

All pigs used in the experiments were Large White x Landrace (live weight between 70 and 90 kg) obtained from the Pig Research and Training Centre, Victorian Institute of Animal Science, Werribee. The pigs were free of the halothane gene. They were transported in groups of 4 in the back of a utility, 1.4 km to the Meat Research and Training Centre (MRTC) 16-20 hours prior to slaughter and housed in pens of 4 under cover. The 4 pigs came from the same pen in the finisher shed and were housed together at the MRTC to minimize fighting. All pigs were of similar genetic background and were produced under similar conditions.

Pigs were stunned using 90% carbon dioxide in air for 1.8 minutes, using a Butina (Denmark) dip lift carbon dioxide stunning unit, unless otherwise stated and were then exanguinated within 40 seconds of stunning using conventional methods. All pigs were shackled on the right leg and were placed in a scald tank for 8 minutes at 60°C at 4-5 minutes post slaughter. The total slaughtering and carcass dressing process took on average 20 ± 5 minutes. When electrical stunning was employed, head to brisket tongs were used while the pigs were restrained in a V-restrainer. The tongs passed 1.3 amps for 4 seconds with variable voltage to achieve constant current. Hot carcass weights (head off) and fat depth at the P₂ site using an introscope were measured within 30 minutes of exanguination. Unless otherwise stated, the carcasses were split and chilled at an air temperature of -2°C to 5°C, air speed 4m/sec.

The lambs used in experiment 9 were purchased from a commercial sale yard and had an average live weight of 50 ± 8 kg. After being held in lairage at the MRTC for 18 hours prior to slaughter, the lambs entered a V-restrainer and were stunned using 200 volts and 1 amp for 4 seconds and were slaughtered using conventional methods. Hot carcass weights and fat depth measurements at the GR site were obtained within 30 minutes post exanguination.

When electrical stimulation was used for pigs, low voltage electrical stimulation was applied at 4 minutes post exanguination using a rectal probe and stick wound clip. A

square, bipolar waveform was applied, providing 200 mA peak to peak with a frequency of 14Hz. Stimulation was either applied for 15 seconds (short) or 60 seconds (long) as specified. The same low voltage electrical stimulation specifications were used for lambs but the stimulation was applied for a duration of 50 seconds.

2.2 MEAT QUALITY

2.2.1 pH and temperature determination

The pH of muscle samples was determined using a portable pH meter (Jenco Electronic Ltd, model 6009) fitted with a polypropylene spear type gel electrode (Ionode IJ421, Brisbane, Queensland). An average of two readings per site were measured, unless otherwise stated, and calibration was performed using pH 4.0 and 7.0 buffers and pH measurements were conducted with temperature compensation. Temperature was determined using a temperature probe attached to the Jenco portable pH meter. Rigor mortis was defined as 60 minutes after a pH < 5.8 was reached except when the pH failed to drop over a two hour interval.

2.2.2 pH determination - Iodoacetic acid method:

One gram muscle samples were collected from the *M. longissimus thoracis et lumborum* at the 5th rib using a drill and muscle corer attachment, frozen in liquid nitrogen and stored at -80°C for 5 days prior to pH determination. Frozen samples were diluted 1:10 (weight:volume) with 5mM iodoacetic acid (pH 7.0) (Bendall, 1978) and macerated in a “Stomacher Lab-Blender 400” for approximately 7 minutes before the pH was measured using a portable Jenco pH meter and glass electrode (Ionode IJ421, Brisbane, Queensland).

2.2.3 Colour

Surface colour (CIE- L^* a^* b^* ; CIE, 1976) was measured in triplicate on a freshly cut surface using a Minolta Chromameter (model CR-200, 2° standard observer, D_{65} lighting and 8 mm aperture in the measuring head). The chromameter was calibrated with a white tile (Y 94.5, x 0.3131, y 0.3201) for pork and measurements were conducted after a 10 minute bloom time at 8°C. For lamb surface colour measurements, samples were bloomed for 30 minutes and the chromameter was calibrated with a red tile (Y 15.6, x 0.446, y 0.313).

2.2.4 Drip Loss

Drip loss was measured using the method of Honikel *et al.* (1986). An 80 ± 5 grams muscle sample was trimmed of fat and connective tissue and suspended in a plastic bag for 48 hours at 2°C. Drip loss was expressed as a percentage of total weight loss during suspension.

2.2.5 Surface exudate

Surface exudate was measured using the method of Kauffman *et al.* (1986). Muscle samples from the 7th rib were freshly cut and after 10 minutes, a 45 mm ashless filter paper (Schleicher and Schuell, Ref. No. 300204) was firmly placed on the surface, removed within 2 seconds and weighed to determine total fluid absorbed. Percentage drip loss was determined by the following equation - %exudate = $-0.1 + 0.06(\text{mg fluid})$.

2.2.6 Dimensional change

The dimensional change of muscles was determined by removing the LTL from its attachment to the ribs then placing 2 pins 10 cm apart at a distance of 5 cm from the cranial end of the muscle. The length between the pins was measured 60 minutes after a pH < 5.8 was reached. The dimensional change was expressed as the difference in length relative to the initial length. No allowance was made for the fibre direction.

2.3 CATHEPSIN ACTIVITY:

2.3.1 Isolation of total cathepsins:

Extraction of total lysosomal enzymes from the muscle was performed using a method adapted from Etherington *et al.* (1987). Five \pm 0.2 grams of muscle was trimmed of fat and connective tissue, minced with scissors and homogenized in 40 ml of ice cold extraction media (50 mM sodium acetate buffer (pH 5.0), 1 mM EDTA and 0.2% (v/v) Triton X-100). The sample was stirred continuously using a magnetic stirrer for several hours on ice, frozen overnight at -20°C and after thawing was centrifuged at 25 000 g for 20 minutes to remove the muscle fibres. The supernatant was removed and kept on ice for immediate determination of cathepsin B, B+L and D activity.

2.3.2 Cathepsin B and B+L:

Cathepsin B and B+L activity was measured by putting supernatant (10 µl) into incubation buffer (0.75 ml) consisting of 100 mM sodium acetate, 5 mM dithiothreitol, 1 mM EDTA, pH 5.5 incubation buffer and 40 µM methylcoumarylamide substrates in dimethyl-sulfoxide as described by Bechet *et al.* (1986). N-CBZ-L-arginyl-L-arginine-7-amido-4-methylcoumarin (Z-Arg-Arg-NMec) was used as the substrate for cathepsin B and N-CBZ-phenylalanyl-arginine-7-amido-4-methylcoumarin (Z-Phe-Arg-NMec) was used as the substrate for cathepsins B+L.

Incubation buffer was pre-incubated at 37°C for 5 minutes with the supernatant and the assays were initiated by the addition of 0.25 ml of the substrate. The reactions were stopped after 15 minutes by adding 3 ml of termination solution (10 mM sodium acetate, 25 mM acetic acid buffer pH 4.3, 30 mM sodium chloroacetate).

The concentration of product was measured using a LS-50 Luminescence spectrometer (Perkin-Elmer) using the fluorescence function with excitation at 360 nm and emission at 460 nm. The zero was set using the termination solution and a value of 100 was set using 1 µM aminomethyl coumarin. Termination solution added to the incubation buffer prior to the addition of enzyme thereby inactivating the enzyme were used as controls. Cathepsin activity was expressed as 1 unit of enzyme releases 1 µmol of product per minute.

2.3.3 Cathepsin D:

The activity of cathepsin D was measured using haemoglobin as the substrate at pH 3.8 incubated at 37°C as described by Zeece and Katoh (1989). The assay was established by adding in order 125 µl of well dialyzed 8% haemoglobin, 125 µl of 1 M sodium formate, pH 3.8, and 0.5 ml of sample. The reaction was terminated after 60 minutes by the addition of 0.5 ml of 8% TCA and centrifuged at 11 000 g for 3 minutes. For the controls, TCA was added to the haemoglobin prior to incubation with the enzyme.

Cathepsin D activity was determined by measuring the amount of TCA soluble peptides using the method described by Barrett and Heath (1977) using a modified

Lowry procedure. 0.5 ml of the TCA solution was added to 2.5 ml of modified alkaline copper reagent and left to stand at room temperature for 10 minutes before 0.25 ml of diluted Folin-Ciocalteu reagent was added. Samples were vortexed and left to stand for 30 minutes at room temperature. The absorbance was read at 750 nm (Hitachi U-2000 Double Beam Spectrophotometer) and 1 unit of activity was defined as an increase of 0.1 absorbance units at 750 nm per 60 minute of incubation at 37°C.

2.4 WARNER-BRATZLER PEAK SHEAR FORCE AND COOKING LOSS

The tenderness of samples from the *M. longissimus thoracis et lumborum* was determined using the method of Moller and Vestergaard (1986). Fresh samples, that is no prior freezing (100 ± 5 g sample for pork and 80 ± 5 g for lamb) were trimmed of all fat and connective tissue, weighed, placed in plastic bags, cooked in an 80°C waterbath for 60 minutes, cooled under running water for 30 minutes, mopped dry, reweighed and held at 2°C over night. Cooking loss was determined as the weight loss during cooking expressed as a proportion of initial weight. A water temperature of 80°C was used for cooking to obtain a rapid increase in temperature to prevent further changes in tenderness during cooking.

After storage overnight at 2°C, five 1.0 x 1.0 x approximately 4.0 cm rectangular blocks were cut from samples along the fiber axis and the Warner-Bratzler peak shear force measured using a Warner-Bratzler shear blade with a triangular cutting edge attached to an Instron Universal testing Machine. An average peak shear force measurement for each sample was determined from the average of five measurements.

2.5 MYOFIBRIL ISOLATION FOR SDS-PAGE

Myofibrils were isolated using a modified method of Swartz *et al.* (1993). Two \pm 0.5 grams of meat was minced with scissors and added to 15 ml of ice cold rigor buffer (10 mM imidazole, 75 mM KCl, 2 mM EGTA, 2 mM MgCl₂, 2 mM NaN₃, pH 7.2) and Polytron homogenized using 3 x 4 sec bursts. The sample was then diluted to 40 ml with rigor buffer, homogenized in a glass Dounce homogeniser with a B-pestle for 50 strokes and centrifuged for 15 minutes at 3 000 g at 4°C. The pellet was resuspended in 40 ml of rigor buffer, Dounce homogenized with the A-pestle for 50 strokes, filtered through cheese cloth and centrifuged for 15 minutes at 3 000 g at

4°C. The pellet was resuspended with rigor buffer containing 0.5% Triton X-100 and centrifuged for 15 minutes at 3 000 g at 4°C. This step was repeated another two times before the pellet was resuspended in rigor buffer and centrifuged a final time for 10 minutes at 3 000 g. The final pellet was resuspended in 15 ml of rigor buffer glycerol mixture (50% glycerol) containing 1 mM dithiothreitol (DTT) by Dounce homogenizing with the B-pestle before being frozen at -20°C.

2.6 SDS-PAGE

One ml of myofibrils suspended in 50% glycerol-rigor buffer was washed free of glycerol-rigor buffer by dilution with 10 volumes of rigor buffer and centrifuged in a microcentrifuge for 15 sec. The washing and centrifuging was repeated four times. After the final dilution, the concentration of myofibrils was determined using the biuret assay (Gornall *et al.*, 1949) and adjusted to a concentration of 4 mg/ml with rigor buffer. Diluted myofibril samples were mixed 1:1 with electrophoresis sample buffer (8 M urea, 2 M thiourea, 3% (w/v) SDS, 75 mM DTT, 25 mM Tris-HCl pH 6.8; Warner *et al.*, 1997) before being heated at 100°C for 4 minutes. The samples were then subjected to SDS-PAGE. Ten percent mini gels (pH 9.3, 10% acrylamide (w/v), 0.06% bis-acrylamide (w/v)) were used as described by Fritz *et al.* (1989). Ten µl of 4 mg/ml samples were applied to each lane with a microsyringe and a 40 mA constant current applied until the tracking gel reached the bottom of the gel. Gels were placed in staining solution (0.5 g Coomassie Brilliant Blue, 500 ml methanol, 400 ml water, 100 ml acetic acid) for 15 minutes, and then destained in 10% methanol, 7.5% acetic acid. Gels were dried by placing them between two cellulose sheets and drying on a gel dryer for several days. All samples were tested and a representative sample is presented in the results for each treatment.

2.7 SARCOMERE LENGTH

Sarcomere length was determined using a helium - neon laser similar to that described by Ruddick and Richards (1975). Thin slices of muscle were cut parallel to the muscle fiber direction and placed between 2 microscope slides. The slide was then placed horizontally in the path of a vertically orientated laser beam to give an array of diffraction bands on the screen. Sarcomere length was calculated using the following equation:

$$\text{sarcomere length (mm)} = \frac{0.635}{\sin (\arctan(d/75))}$$

where d is the distance (mm) of the diffraction spot from the centre of the screen. The average of 20 sarcomeres per muscle sample was used. The average distance from the center to the band was determined using the distance of both the inside and the outside of the band to improve accuracy.

2.8 MYOFIBRILLAR FRAGMENTATION INDEX

The myofibril fragmentation index was determined using modifications to the method described by Culler *et al.* (1978). Two grams of muscle that had previously been frozen in liquid nitrogen and stored at -80°C was trimmed of fat and connective tissue, scissor minced and polytron homogenized for 30 sec in 40 ml of ice cold isolating media (100 mM potassium chloride, 20 mM potassium phosphate, 1 mM EDTA, 1 mM magnesium chloride and 1 mM sodium azide).

The homogenate was centrifuged at 1000 g at 2°C for 15 minutes and the sediment resuspended in 20 ml of isolating media with a stirring rod, centrifuged again at 1000 g at 2°C for 15 minutes and the supernatant decanted.

The pellet was resuspended in 6 ml of isolating media at 2°C and passed through a polyethylene strainer (18 mesh) to remove connective tissue and debris. An additional 5 ml of isolating media was added to facilitate passage of myofibrils through the strainer.

Protein concentration of the myofibril suspension was determined by the biuret method (Gornall *et al.*, 1949) and the suspension diluted to a protein concentration of 0.5 ± 0.05 mg/ml. The actual protein concentration was again measured using the Biuret method to ensure a protein concentration of 0.5 ± 0.05 mg/ml was achieved. The absorbance of the myofibril suspension was measured immediately at 540 nm and multiplied by 200 to give a myofibril fragmentation index.

2.9 PROTEIN SOLUBILITY

2.9.1 Method 1

The solubility of the sarcoplasmic proteins was determined using the method described by Warner *et al.* 1997). A 1 ± 0.2 g samples was homogenized on ice in

10 ml of ice cold 0.025 M potassium phosphate (pH 7.2) using 3x4 sec bursts. The solubility of total proteins was determined by homogenizing 1 ± 0.2 g samples in 20 ml of ice cold 1.1 M potassium iodide/ 0.1 M potassium phosphate (pH 7.2). The samples were left to stand overnight at 2°C and centrifuged at 1 500 g for 20 minutes and the supernatant decanted. All samples were performed in duplicate.

Protein concentration of the supernatant was determined by the biuret method (Gornall *et al.*, 1949) using BSA as the standard. The concentration of myofibrillar protein solubility was determined as the difference between the total protein solubility and the sarcoplasmic protein solubility.

2.9.2 Method 2

Sarcoplasmic protein solubility was determined from the 2 g of muscle samples used to isolate myofibrils for SDS-PAGE. The rigor buffer supernatant after the initial centrifugation was analysed for the sarcoplasmic protein content using the biuret method.

Myofibrillar protein solubility was determined by diluting 50 mg of isolated myofibrils in rigor buffer with an equal volume of 2.2 M potassium iodide/ 0.2 M potassium phosphate (pH 7.2). The samples were stored at 2°C for 24 hours, centrifuged for 10 minutes at 3 000 g before the protein concentration of the supernatant was determined by the Biuret method (Gornall *et al.*, 1949). The myofibrillar protein solubility was determined to be the concentration of protein suspended after standing 24 hours.

2.10 PURGE

Purge was determined as the amount of moisture lost from the meat during storage of samples in vacuum bags over specified storage periods. Purge was expressed as the difference in weight between samples into the vacuum bag (prepackaging) and after removal from packaging expressed as a percentage of prepackaging weight.

2.11 SENSORY ANALYSIS

An untrained consumer sensory panel was used to assess the consumer perception of toughness, flavour, juiciness and overall acceptability of pork samples. The caudal end of the *M. longissimus thoracis et lumborum* (250 ± 20 g) was collected 60

minutes after a pH < 5.8 was reached, trimmed of fat and connective tissue, vacuum packaged and stored at 2°C for 2 days until consumer sensory panel assessment.

Samples were cooked in a Rational Combi- Steamer oven (model CCC) set at 160°C until an internal temperature of 70°C was reached as determined using an internal temperature probe. After reaching 70°C, the samples were removed from the oven, wrapped in foil and sliced into 5 mm slices just prior to tasting 10 minutes after cooking. Panelists were provided with water crackers and 50% apple juice to cleanse their palate between each sample.

The taste panel comprised 20 people consisting of staff and students based at the Victorian Institute of Animal Science. Each panelist tasted 4 samples in each session and 2 sessions were run per day. A total of 6 sessions were run with 8 samples being evaluated at each session thereby each sample was evaluated by 10 panelists. Treatments were blocked so that each treatment was represented once in each session and samples were randomly allocated to panelists to ensure that every combination of samples was represented. Samples were scored using an unstructured scale with anchors at each end for toughness (1 = extremely tender, 100 = extremely tough), flavour (1 = dislike extremely, 100 = like extremely), juiciness (1 = extremely dry, 100 = extremely juicy) and overall acceptability (1 = dislike extremely, 100 = like extremely). The average response of ten panelists was used in the analysis of variance.

The panel was requested to completed the following questions:

Age (years)	8-12 []	13-17 []	18-24 []	25-30 []
	31-39 []	40-49 []	50-55 []	56-60 []
Gender	Male []		Female []	

How often do you consumer each of the following meat dishes?

	Once a week	Once a fortnight	Once a month	Once every 3 months	Never
Pork in stir fry	[]	[]	[]	[]	[]
Roast pork	[]	[]	[]	[]	[]
Beef as medium	[]	[]	[]	[]	[]

steak

Beef as well [] [] [] [] []

done steak

Chicken in stir [] [] [] [] []

fry

Roast chicken [] [] [] [] []

Taste the product:

1. What do you think of its tenderness?

Extremely
tender

Extremely
tough

2. Liking of flavour:

Dislike
extremely

Like
extremely

3. Overall juiciness:

Not juicy

Very juicy

4. Overall acceptability:

Dislike
extremely

Like
extremely

Comments:

3. TENDERNESS AND MEAT QUALITY OF PORK *M. LONGISSIMUS THORACIS ET LUMBORUM* AFTER ACCELERATED BONING.

3.1 INTRODUCTION

Accelerated boning of pork carcasses is one approach to reducing the costs of pork boning while maintaining meat quality. Accelerated boning of meat carcasses involves the removal of primals or cuts from the carcass pre-rigor (van Laack, 1989) while conventional boning involves the removal of primals from the carcass after rigor mortis has set in. Accelerated boning enables production costs to be reduced due to lower labour requirements due to reduced handling of the meat and faster boning times, a reduction in chiller space and energy input and increased product turnover due to faster chilling of accelerated boned products (Cross and Seidman, 1985; Powell *et al.*, 1982). Accelerated boning of pork has been slow to be employed on a wide scale in Australia, largely due to the logistics of changing to accelerated boning and also the potential detrimental impact on tenderness because of the rapid drop in muscle temperature that occurs when muscles are removed pre-rigor. Due to the majority of accelerated boning studies being on beef, studies on pork are required to determine if the economic benefits of accelerated boning can be employed with pork without reducing quality and in particular tenderness.

The temperature of meat after slaughter has a large effect on the time to development of rigor mortis, cold shortening and meat tenderness. A major problem experienced with accelerated boned meat is cold shortening, which can result in up to 30% increase in toughness in pork cuts (Cross and Seidman, 1985) and an increase in drip loss (Honikel, 1986). Cold shortening is due to a rapid release of calcium from the sarcoplasmic reticulum and mitochondria, as a result of the rapid temperature reduction due to the lower binding capacity for calcium at low temperatures (Locker, 1985; Pearson and Dutson, 1985). The calcium ions are not reabsorbed by the mitochondria due to the decreasing ability of the mitochondria to bind calcium with the decrease in temperature (Martinosi and Feretos, 1964). Thus, the binding of the calcium to the troponin system in the thin filaments releases the blocking effect of tropomyosin on actin, permitting the interaction between actin and

myosin (Pearson, 1986; Locker and Hagyard, 1963). This results in contraction and a reduction in sarcomere length, which increases meat toughness (Honikel *et al.*, 1986). Under conventional boning conditions, cold shortening is minimised or prevented by slower cooling of the carcasses before rigor sets in and by the muscles being restrained by attachment to bones and tendons within the carcass (Locker and Hagyard, 1963).

To avoid cold shortening in pork undergoing accelerated boning, careful monitoring of the rates of chilling must occur to prevent toughening and increased drip loss. To avoid cold shortening, a pH of 6.0 must be reached while the muscle temperature is above 10°C (Honikel and Reagan, 1987; Locker and Hagyard, 1963). If the temperature is less than 10°C the potential for cold shortening increases thereby potentially decreasing tenderness and increasing drip loss. However, research has shown that low temperatures may be used without inducing cold shortening. Honikel (1986) observed a storage temperature of 10°C resulted in minimal sarcomere length shortening for the *M. cleidocipitalis*. Fischer *et al.* (1980) reported that even at 4°C minimal sarcomere length shortening occurred with the *M. longissimus thoracis et lumborum* (LTL) along with minimal drip loss.

Furthermore, high chilling temperatures also need to be avoided due to the potential of heat shortening and protein denaturation. Heat shortening occurs at temperatures greater than 35°C prior to the onset of rigor (Judge *et al.*, 1989). Heat shortening results from a rapid depletion of ATP due to higher temperatures accelerating ATP disappearance, resulting in extensive muscle contraction (Judge *et al.*, 1989).

In contrast to the negative impact of accelerated boning on tenderness, the use of accelerated boning for pork muscles has the potential to improve other meat quality traits. Both less pale meat colour (van Laack, 1989) and reduced drip loss (van Laack and Smulders, 1992) have been reported following accelerated boning of pork muscle. The conditions that occur during accelerated boning are ideal to minimise PSE. PSE pork is a result of a rapid rate of pH decline while muscle temperature is still high, leading to protein denaturation. This protein denaturation results in pork with pale surface colour and excessive drip loss. The rapid fall in muscle temperature that occurs during accelerated boning can reduce the rate of pH decline. The reduced rate of pH decline thereby reduces protein denaturation and reduces the

occurrence of PSE. The darker colour seen in pork after accelerated boning has also been reported in beef (Shaw and Powell, 1995) and can be attributed to the slower pH decline and increased water holding capacity. However, decreases in water holding capacity of accelerated boned muscles have also been reported in both pork and beef (Honikel *et al.*, 1986; Iversen *et al.*, 1994; Shaw and Powell 1995) and can be attributed to the reduction in sarcomere length due to the occurrence of cold shortening. The reduction in sarcomere length reduces the intermolecular space available for the water thereby decreasing the water holding capacity of the muscle.

Conflicting results are presented in the literature on the extent of cold shortening in pork and the effects of cold shortening on tenderness and drip loss for pork that has undergone accelerated boning (van Laack and Smulders, 1992; Iversen *et al.*, 1994; Dransfield and Lockyer, 1985). Due to these conflicting findings additional research is required to investigate the influence of accelerated boning on pork meat quality and tenderness under Australian conditions. In Australia, the typical methods of stunning employed for pigs are carbon dioxide and electrical stunning. As differences in the rate of pH decline have been reported with carbon dioxide and electrical stunning (Channon *et al.*, 1997), the effects of accelerated boning of pork in Australia could be further influenced by the method of stunning employed.

When taking samples for meat quality (including tenderness) assessment, care must be taken to avoid naturally occurring variations along the muscle that may alter results obtained. The obvious sources of variation that could influence treatment responses are between animals and between different muscles (Mackey and Oliver, 1954) due to variations in both fibre type and collagen content. This naturally occurring variation has lead to experiments being conducted using a large number of animals to represent the population and selecting specific muscles for investigation. Very few investigations take into consideration the uniformity of fibre type and collagen content or lack of it throughout one particular muscle. If the *M. longissimus thoracis et lumborum* (LTL) were similar along its length, it would allow samples to be collected without regard to position, simplifying sampling.

Significant variations in Warner-Bratzler peak shear force (WBSF) between different anatomical locations within individual muscles have been reported in some studies (Weir, 1953; Kinsman, 1961; Ginger and Weir, 1958; Batchner and Dawson, 1960;

Taylor *et al.*, 1961 and Mackey and Oliver, 1954) while other studies have reported no differences (Wheeler and Koohmaraie, 1994; Moller and Vestergaard, 1986). Conflicting views on the most tender section within beef and pork *longissimus* and *semimembranosus* muscles have been reported (Weir, 1953; Kinsman, 1961; Ginger and Weir, 1958; Batcher and Dawson, 1960; Taylor *et al.*, 1961 and Mackey and Oliver, 1954). Furthermore, conflicting reports have previously been presented on whether variation in tenderness along the LTL does in fact occur (Wheeler and Koohmaraie, 1994; Moller and Vestergaard, 1986).

The differences in WBSF between anatomical locations may be due to different stunning and boning methods. The majority of the studies mentioned previously have not mentioned the method of stunning or chilling employed. As the method of stunning can alter the rate of rigor development along the LTL (Channon *et al.*, 1997), this could potentially alter WBSF along the LTL muscle. Differences in WBSF along the muscle may be due to variations in the level of shortening and variations in proteolytic activity due to different pH and temperature relationships occurring along the muscle. It was proposed to use carbon dioxide stunning in the experiments, thus variations in WBSF along the LTL may potentially occur. Furthermore potential variations in chilling rates between experiments may also influence the rate of pH decline and thus WBSF due to the interaction between chilling and pH decline rates. Thus it is important to identify how WBSF may be influenced by both carbon dioxide stunning and the chilling regime employed in this research.

It is well established that meat tenderness undergoes changes post mortem (Koohmaraie *et al.*, 1995). Immediately after slaughter meat is tender but due to muscle shortening during the onset of rigor mortis, the meat becomes tougher. Simultaneously, the process of tenderisation is initiated due to proteolysis beginning shortly after slaughter with the activation of the tenderising enzymes. The conditions during rigor development are one of the most important factors initiating and controlling tenderisation. As variations in rigor conditions such as the rate of pH and temperature decline can alter muscle structure, the release of calcium ions and the activity of proteolytic enzymes (Dransfield, 1994), the rate of tenderisation and ultimate tenderness are thus highly dependent upon these early conditions.

Variations in the rate of ageing of pork with post slaughter storage have been reported in the literature. For example, Harrison *et al.* (1970) and Buchter and Zeuthen (1971) observed an increase in tenderness up to the sixth and eighth days post mortem respectively while Bennett *et al.* (1973) found that ageing more than one to two days did nothing to improve the palatability of pork. Feldhusen and Kuhne (1992) found that optimum shear force values were attained at two to three days post mortem. Dransfield *et al.* (1980-81) found on average 50% of the tenderisation of pork has been observed to occur in two days compared to 4.2 days for beef and veal. Thus, further investigation is required to investigate the ageing rate of pork under the conditions employed throughout this work.

The objectives of the work presented in this chapter are as follows.

1. Experiment 1: Variation in tenderness along the loin

To investigate the variation in tenderness and cooking loss between segments of the *M. longissimus thoracis et lumborum* at 1 day post slaughter. If differences in tenderness are shown, careful attention would then need to be employed in future experiments to ensure that these differences did not influence the results obtained.

2. Experiment 2: Rate of ageing of pork

To investigate the rate of ageing of pork *M. longissimus thoracis et lumborum* by studying the WBSF changes with time. As a result the best ageing period could be determined in order to collect aged samples once 80% of ageing had occurred in subsequent experiments.

3. Experiment 3: Tenderisation of pork loin after accelerated boning

To investigate the effect of accelerated boning and 0°C chilling on the tenderness and overall meat quality of pork *M. longissimus thoracis et lumborum*. Therefore, it can be determined if cold shortening does occur with chilling at 0°C and the impact this has on sarcomere length, drip loss and tenderness.

This research was carried out using standardised genetics (Large White x Landrace finisher female pigs), transport, handling, lairage, stunning and slaughtering conditions.

3.2 METHODOLOGY

3.2.1 Experiment 1: Variation in tenderness along the loin

Eight Landrace x Large White female pigs were slaughtered conventionally on one day and within 30 minutes post slaughter the carcasses were split and the sides placed in chillers at 2°C for chilling overnight. The pH and temperature of the LTL muscle was measured at 40 minutes post slaughter between the 5th and 6th lumbar vertebrae. The LTL from both sides was removed at 1 day post slaughter, cut into twelve 100 g segments and numbered from the cranial end as indicated in figure 3.1. The twelve segments were cooked within 30 minutes of cutting and assessed for cooking loss and WBSF using the methods described in chapter two. Colour, pH, temperature and surface exudate using the filter paper method were also assessed at 1 day post slaughter at the 12/13th rib using methods described in chapter two.

3.2.2 Experiment 2: Rate of ageing of pork

Four Landrace x Large White female pigs were slaughtered conventionally on one slaughter day and within 30 minutes post slaughter the sides were placed in the chiller at 2°C. The pH of the LTL was measured at the 5th thoracic vertebrae (5TV), the 13th thoracic vertebrae (13TV) and the 5th lumbar vertebrae (5LV) every 30 minutes from slaughter to determine when rigor mortis had set in as outlined in chapter two. Rigor mortis was presumed to have occurred when a pH < 5.8 was reached at all 3 sites. Once the side was considered to have attained rigor mortis, it was left for 1 hour before the LTL was removed and cut into twelve 150 gram samples as outlined in Figure 3.1. These samples were randomly allocated to an ageing period, vacuum packaged and stored at 2°C. WBSF and cooking loss was determined at rigor, 0.5, 0.75, 1, 1.25, 1.75, 2.5, 3, 4, 5, 7 and 9 days post slaughter. All methods are described in chapter two.

3.2.3 Experiment 3: Tenderisation of pork loin after accelerated boning

Eight Landrace x Large White female pigs were slaughtered conventionally on one slaughter day, split in half and the sides randomly allocated to accelerated (AB) or conventional boning (CB). The CB treatment involved chilling the sides for 1 day at 2°C. For the AB treatment the LTL muscle was removed within 30 minutes post

slaughter, trimmed of subcutaneous fat and chilled in an ice water bath for 6 hours then placed in the chiller at 2°C until 1 day post slaughter. The dimensional change from 30 minutes to 24 hours post slaughter for samples undergoing accelerated boning was determined using the method described in chapter 2. For both treatments, the LTL muscles was cut in half at the 12th rib at 1 day post slaughter and the caudal end vacuum packaged and aged for 4 days at 2°C. The cranial end was used for 1 day meat quality and biochemical assessment.

3.2.3.1 Meat quality measurements

The pH was measured every 30 minutes for the first 3 hours post slaughter then every hour until 6 hours post slaughter and at 1 day post slaughter. The pH was determined using the iodoacetic acid method using 1 ± 0.05 g muscle drill samples as described in chapter two. At 1 day post slaughter, a 80 g sample from the 9th rib of the cranial end of the LTL muscle was used to assess meat colour, drip loss, cooking loss and WBSF using the methods outlined in chapter two. A 50 ± 10 g sample was also collected at 1 day post slaughter, frozen in liquid nitrogen and stored for 1 month at -80°C for biochemical measurements.

At 4 days post slaughter, the caudal end of the LTL was removed from the vacuum bag. A 100g sample was removed from the 13th rib end for measurement of WBSF and cooking loss. Purge and cooking loss was also determined as the moisture lost during storage as outlined in chapter two and a 50 g sample was frozen for biochemical measurements as for the samples collected at 1 day post slaughter.

3.2.3.2 Biochemical measurements

At 1 day post slaughter, a fresh muscle sample was used to measure the activity of total cathepsins B, B + L and D after isolation of the cathepsins as described in chapter two. The frozen samples collected at 1 and 4 days post slaughter were assessed for myofibrillar fragmentation index (MFI), sarcomere length and SDS-PAGE using the methods in chapter two. At 1 day post slaughter, muscle samples were removed for sarcoplasmic and myofibrillar protein solubility using method one outlined in chapter two.

3.2.4 Statistics

The data from the experiments presented in this chapter were analysed using Genstat 5 (Payne *et al.*, 1988).

The rate of pH and temperature decline and rate of WBSF decline for each treatment in the three experiments was determined by fitting the following equation to the average values for each treatment at each time measured using the fit curve function of Genstat 5 (Payne *et al.*, 1988);

$$F_t = F_{\infty} + (F_0 - F_{\infty})e^{-kt} \text{ (Dransfield *et al.*, 1980-81)}$$

where F_t , F_0 and F_{∞} are the WBSF/pH/temperature values at time t , at time zero (at time of slaughter for pH and temperature decline and at time of first rigor for WBSF) and at the completion of ageing/pH/temperature decline respectively and k is the rate constant.

3.2.4.1 Experiment 1: Variation in tenderness along the loin

The LTL was divided up for statistical analysis into sections, portions, grouped portions and halves to determine if variation in WBSF and cooking loss occurred. The sections consisted of the 12 pieces of LTL assessed as outlined in Figure 3.1. Six portions were assessed with the sections grouped according to the following - a = section 1, b = sections 2 and 3, c = sections 4 and 5, d = sections 6 and 7, e = sections 8 and 9, f = sections 10 and 11 and g = section 12. Grouped portions involved the sections grouped as follows - i = section 1, ii = sections 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11 and iii = section 12. Halves involved the sections being grouped as follows - A = sections 2, 4, 6, 8, and 10 and B = 3, 5, 7, 9 and 11. WBSF and cooking loss were analysed using variant components analysis to determine if differences occurred between the sections and between pigs. Analysis of variance was used to determine the difference in WBSF and cooking loss between animals, between grouped portions within animals and halves crossed with portions within grouped portions. Linear and quadratic relationships for cooking loss and WBSF were determined using regression analysis.

3.2.4.2 Experiment 2: Rate of ageing of pork

The rate of pH, temperature and WBSF decline were determined as described above.

3.2.4.3 Experiment 3: Tenderisation of pork loin after accelerated boning

The data in experiment 3 was analysed by ANOVA using a randomised block design. The treatments were randomised between each side of each pig and the data was blocked on pig. Drip loss required log transformation to make the error variation more homogenous with increasing values. The rate of pH decline was determined as described above. Correlation coefficients and regression analysis between sarcomere length, MFI and WBSF were determined. The percentage change from 1 to 4 days post slaughter was determined for WBSF to provide an indication of the level of ageing using the following equation:

$$\% \text{ change WBSF} = \frac{\text{WBSF}_{1 \text{ day}} - \text{WBSF}_{4 \text{ days}}}{\text{WBSF}_{1 \text{ day}}} \times 100$$

3.3 RESULTS

3.3.1 Experiment 1: Variation in tenderness along the loin

The average live weight of the pigs slaughtered was 91 ± 3.2 kg, the average hot carcass weight was 65 ± 3.3 kg and the average P₂ fat depth was 22 ± 5.1 mm.

The mean and standard deviation for pH measured at 40 minutes post slaughter was 6.65 ± 0.192 and at 1 day post slaughter was 5.61 ± 0.217 . The exudate and CIE-L* at 1 day post slaughter was $0.02 \text{ mg} \pm 0.004 \text{ mg}$ and 48.9 ± 2.14 respectively. Using the definition of PSE and DFD described by Warner *et al.* (1993) (PSE L* > 50, drip > 5% and DFD L* < 42, drip < 5% and pH_u > 6.0), no LTL muscles exhibited these conditions.

There was a significant increase in cooking loss from the cranial to caudal end of the LTL (P < 0.05) (Figure 3.2). Sections 10 and 11 had a higher cooking loss than sections 1 to 5 while section 12 had a higher cooking loss than section 1. Cooking loss increased by 6.5 % from the cranial to the caudal end of the LTL. There was no differences in cooking loss between the 2 sides of each pig (P > 0.05) and the between animal variation was smaller than the variation along the LTL.

There was no difference in WBSF between any sections of the LTL (P > 0.05) (Figure 3.2). There was no difference in WBSF between sides of each pig (P > 0.05). There

were differences between the portions ($P < 0.01$) with portion g having a lower WBSF than all other portions and portion d having a higher WBSF than portions b, c, e, f and g. A significant difference in WBSF was observed for the grouped portions ($P < 0.001$) with the caudal end having a lower WBSF than the middle and the cranial end of the muscle but there was no differences between the halves ($P > 0.05$).

3.3.2 Experiment 2: Rate of ageing of pork

3.3.2.1 Sample Description

The average live weight of the pigs was 92 ± 7.3 kg, the average hot carcass weight was 66 ± 5.2 kg and the average P_2 fat depth was 21 ± 3.4 mm. No LTL muscles exhibited either PSE or DFD based on the pH data ($PSE = pH_{45min} < 5.8$).

3.3.2.2 pH and temperature decline

The pH decline with time curves for the three different sites along the LTL can be seen in Figure 3.3 with the cranial end of the muscle (5TV) having a faster pH decline than the middle (12TV) and caudal end (5LV) ($R^2 = 96\%$ for each site, $RSD = 0.04$ to 0.06 , $P < 0.05$). The average time to reach rigor mortis was 6 hours in all three sites. Temperature decline with time post slaughter for the three sites along the LTL was similar at the 5LV and 12 TV sites while it was slower at the 5TV site and could be described by exponential decay curves ($R^2 = 94$ to 97% , $RSD = 1.23$ to 1.75 , $P < 0.05$) which can be seen in Figure 3.4.

3.3.2.3 Cooking loss

There was no change in cooking loss over the 10 day ageing period ($P > 0.05$). The average cooking loss was 33.2% with a standard error of 1.19%.

3.3.2.4 Ageing Rate

The relationship between average WBSF for each time measured and the time post slaughter of pork LTL fitted an exponential decay curve (Figure 3.5):

$$WBSF \text{ (kg)} = 4.08 + 4.14e^{-0.38time} \quad (R^2 = 92.4\%, RSD = 0.35, P < 0.05)$$
 where time = days post slaughter. This equation predicts that 50% of the change in

tenderness occurred within 1.8 days post slaughter, 80% of change in tenderness had occurred by 4.2 days post slaughter and 90% within 6.1 days post slaughter.

3.3.3 Experiment 3: Tenderisation of pork loin after accelerated boning

3.3.3.1 Sample Description

The average live weight of the pigs was 93 ± 7.3 kilograms, the average hot carcass weight was 66 ± 3.1 kilograms and the average P₂ fat measurement of 20 ± 3.4 mm. Using the definition of PSE and DFD described by Warner *et al.*, (1993) (PSE L* > 50, drip > 5% and DFD L* < 42, drip < 5% and pH_u > 6.0), one LTL muscle from the conventionally boned treatment exhibited PSE conditions.

3.3.3.2 Rate of pH and temperature decline

The average pH decline of the accelerated boned and conventionally boned muscles can be seen in Figure 3.6. There was no differences in pH between the two treatments at 0.5, 1.5, 2, 2.5, 4, 5 and 6 hours or 1 day post slaughter ($P > 0.05$). However at 1 and 3 hours post slaughter the accelerated boned muscles had a higher pH than the conventionally boned muscles ($P < 0.05$).

The temperature decline data that was measured on a data logger was lost due to a computer failure. In subsequent experiments a temperature of less than 10°C occurred when the pH was still above 6.0 where the same treatments were employed as those here, it is assumed that similar conditions occurred in this study.

3.3.3.3 Meat colour and drip loss

Accelerated boning influenced the LTL meat quality characteristics at 1 day post slaughter (Table 3.1). Drip loss was increased by accelerated boning of the muscles relative to the conventionally boned samples ($P < 0.05$). Samples undergoing accelerated boning also had a lower L*, a* and b* values compared with the conventionally boned samples ($P < 0.05$). The correlation coefficients between L* and drip loss, and between sarcomere length and drip loss were not significant ($P > 0.05$) (results not presented).

3.3.3.4 *Warner-Bratzler peak shear force, dimensional change, sarcomere length and myofibrillar fragmentation index*

WBSF was higher for the accelerated boned samples at both 1 and 4 days post slaughter ($P < 0.01$) (Table 3.2). Ageing occurred in both treatment groups as indicated by a reduction in WBSF from 1 to 4 days post slaughter with a reduction in WBSF values by 2.2 kg for samples undergoing accelerated boning and 1.4 kg for samples undergoing conventional boning. However, the percentage change in WBSF from 1 to 4 days post slaughter was not influenced by the method of processing ($P > 0.05$) (Table 3.2).

The average dimensional change of the samples undergoing accelerated boning was 15%. The dimensional change calculated for the direction of the muscle fibres was 21%. The dimensional change of the conventionally boned samples was not determined due to the limitations imposed by the skin. Accelerated boning reduced sarcomere length at both 1 and 4 days post slaughter relative to conventionally boned muscles ($P < 0.001$) as seen in Table 3.2.

Accelerated boning significantly reduced the MFI values at both 1 day and 4 days post slaughter relative to conventionally boned muscles ($P < 0.05$) (Table 3.2) indicating that a greater level of myofibrillar fragmentation occurred in the samples following conventional boning.

At 1 day post slaughter there was a correlation between WBSF and sarcomere length ($r = -0.77$, $RSD = 2.35$, $P < 0.001$) and between myofibrillar fragmentation index and sarcomere length ($r = 0.55$, $RSD = 0.21$, $P < 0.05$). At 4 days post slaughter, there was a correlation and a linear relationship between WBSF and MFI ($r = -0.52$, $RSD = 2.1$, $P < 0.01$), and between WBSF and sarcomere length ($r = -0.80$, $RSD = 1.46$, $P < 0.001$).

3.3.3.5 *Cathepsin activity, protein solubility and protein degradation*

Accelerated boning had no effect on total cathepsin B, B+L or D activity at 1 day post slaughter ($P > 0.05$) (Table 3.3) indicating that the method of boning did not influence the activity of these enzymes. Accelerated boning also had no effect on the solubility

of sarcoplasmic or myofibrillar proteins ($P > 0.05$) (Table 3.3) indicating that the method of boning did not alter the level of protein denaturation.

A representative SDS-PAGE gel for the different treatments can be seen in figure 3.7. A comparison of protein degradation in myofibril samples from 1 to 4 days post slaughter using SDS-PAGE illustrates that ageing occurred in the conventionally boned muscles over this time period as indicated by changes to the nebulin and troponin T bands. Breakdown of nebulin can be observed to occur in the samples following conventional boning by 4 days post slaughter with the single nebulin band disappearing by 4 days post slaughter and the appearance of a triplet but not in the samples following accelerated boning. Troponin T does not appear to have disappeared in the 4 day samples following accelerated boning but it has in the muscles following conventional boning with the formation of a 30 kDalton band.

3.3.3.6 Cooking loss and purge

Accelerated boning increased cooking loss at both 1 and 4 days post slaughter relative to the muscles undergoing conventional boning ($P < 0.001$) as seen in Table 3.4. Purge was also increased ($P < 0.01$) in muscles that had undergone accelerated boning relative to the conventionally boned muscles (Table 3.4).

3.4 DISCUSSION

3.4.1 Experiment 1: Variation in Warner-Bratzler peak shear force and cooking loss along the loin

3.4.1.1 Warner Bratzler peak shear force

Similar WBSF values in experiment 1 were obtained to those previously reported for pork loin at 1 day post slaughter (Jones *et al.*, 1993; Feldhusen and Kuhne, 1992). Moller and Vestergaard (1986) reported WBSF values of 4.3 to 6.2 kg for pork samples collected at 1 day post slaughter using the same Warner Bratzler method as used in experiment 1. The slightly lower values observed by Moller and Vestergaard (1986) may be attributed to the samples being frozen prior to testing with freezing reported to lower WBSF (Ferrier and Hopkins, 1997). Furthermore, Moller and Vestergaard (1986) cooked their samples in a 0.9% NaCl solution at 80°C for 25

minutes which may have further reduced tenderness as reported by Alarcon-Royo and Dransfield (1995).

Experiment 1 examined the variation in WBSF along the loin using analysis of variance and variant components analysis. No variation in WBSF between sections was observed using variant components analysis. This consistency in tenderness along the LTL was also reported by Wheeler and Koochmaraie (1994) for beef who reported that sample location within the *longissimus* was not important provided that the samples for WBSF testing were cut parallel to the fibre orientation, the same cutting direction used in this experiment. Similar results for beef LTL were reported by Sharrah *et al.* (1965) who reported no significant differences in WBSF between the proximal and distal ends. As no variations in tenderness were observed along the LTL, this indicates that anatomical location of WBSF samples does not need to be considered when taking samples.

In contrast, significant differences in WBSF were observed when analysis of variance was performed with position as a fixed effect on the WBSF data in experiment 1. This indicated that the middle portion was tougher than the end sections, a similar result to that observed by Moller and Vestergaard (1986) in pork loin. This analysis however assumes the residuals are equally correlated within pigs across the LTL. Therefore, care must be taken when looking at these results. The experiment contained too few animals and too many sites to reliably test thus variant component analysis is a more reliable test.

In contrast to these results several researchers have found variations in WBSF along muscles for beef and pork (Moller and Vestergaard, 1986; Smith *et al.*, 1969; Hedrick *et al.*, 1968; Romans *et al.*, 1965; Henrickson *et al.*, 1964; Mackey and Oliver 1954; Ginger and Weir, 1958; Taylor *et al.*, 1961). Smith *et al.* (1969) reported that anatomical location of beef samples should be carefully controlled to prevent locational differences affecting tenderness measurements. They found a gradient exists over the cross section of the LTL with the medial position being more tender than the lateral position thereby core samples should be taken from as many and as varied a position as is feasible. Smith *et al.* (1969) found that beef steaks adjacent to the 12th thoracic vertebrae were more tender than those nearer the 10th or 11th thoracic vertebrae. Furthermore, Romans *et al.* (1965) and Henrickson *et al.* (1964)

indicated that beef LTL steaks from the 9th thoracic vertebrae were more tender than those from the 11th thoracic vertebrae. Studies by Moller and Vestergaard (1986) revealed that the average shear force values at the 1st to 4th lumbar vertebrae and 9th to 11th rib were lower than the values at the 12th to 15th rib.

The differences in tenderness along the LTL reported in some of the literature but not seen in experiment 1 may be attributed to a number of factors. Variations in the rate of pH decline along the muscles may have occurred in other experiments thereby altering the tenderness along the muscle with differences in pH decline between and within muscles being previously reported by Marsh *et al.* (1980-81). The method of cooking may also help to explain the differences seen in experiment 1 in relation to the literature. The beef samples were either braised (Taylor *et al.*, 1961), roasted (Smith *et al.*, 1969; Mackey and Oliver, 1954) or cooked in fat (Hedrick *et al.*, 1968; Gould *et al.*, 1965) while the pork samples of Moller and Vestergaard (1986) were cooked in NaCl solution. These differences in cooking method could affect both the connective tissue and myofibrillar component of toughness thus altering the tenderness results (Cross, 1987). Furthermore differences in stunning procedures, fibre type distribution, intramuscular fat and method of analysis may also help to explain the differences in the results seen in experiment 1 compared to literature reports as all these factors can alter tenderness. The method of stunning and fibre type distribution are important as they may influence the rate of pH decline along the muscle thus altering the tenderness and the level of intramuscular fat will also cause variations in the tenderness values obtained. Furthermore the fibre type will influence the level of tenderising enzymes and their inhibitors (Klont *et al.*, 1998; Dransfield, 1994).

3.4.1.2 Cooking Loss

Increases in cooking loss occurred from the cranial to caudal ends of the pork LTL in experiment 1 which is consistent with other research (Moller and Vestergaard, 1986; Smith *et al.*, 1969). Cooking loss variations along the LTL were also seen by Smith *et al.* (1969) who reported increases in cooking loss at anterior locations of beef LTL with steaks near the 12th thoracic vertebrae having lower cooking loss than those near the 10th or 11th thoracic vertebrae. Differences in cooking loss due to position in pork LTL was also observed by Moller and Vestergaard (1986). Moller and

Vestergaard (1986) reported cooking losses of 43.5%, 42.7% and 41.6% respectively with pelvic suspension at 1st to 4th lumbar vertebrae, 12th to 15th rib and 9th to 11th rib respectively. These cooking loss values compare to 35.3%, 31.6% and 30.4% for the three sites respectively in this study.

In experiment 1, a linear increase in cooking loss was observed from the cranial to the caudal end of the muscle. These differences in cooking loss may be due to differences in intramuscular fat along the length of the muscle and due to differences in the fibre types along the muscle. Differences in the composition of the LTL muscle have been reported with Kiessling and Hansson (1983) reporting the LTL muscle to contain 84% fast twitch glycolytic, 10% fast twitch oxidative glycolytic and 6% slow twitch oxidative while Hunt and Hedrick (1977) reported the LTL to contain 46% fast twitch glycolytic, 25% fast twitch oxidative glycolytic and 29% slow twitch oxidative muscle fibres. Due to the different glycolytic rates of the different fibre types, the rate of rigor development could be altered and thus the level of sarcomere shortening may be altered which will influence the water holding capacity of the muscle.. Although variations in both cooking loss and WBSF were observed in pork LTL muscle by Mackey and Oliver (1954), no linear trends for cooking loss were observed unlike that seen for cooking loss in experiment 1. However, when Mackey and Oliver (1954) examined animals individually rather than as a group, linear increases in cooking loss from rib to loin end were seen in two thirds of the pigs suggesting that position must be considered when allocation to a particular treatment occurs.

Overall, from experiment 1 it can be concluded that under conditions of this experiment although there was little variation in WBSF along the LTL, cooking loss did increase from the cranial to the caudal end. The conditions of the experiment included carbon dioxide stunning and standardised handling, transport, lairage, carcass boning rate and chilling rate. To avoid differences in cooking loss influencing results in future experiments, the variation was accounted for during randomisation and allocation of samples to each ageing period.

3.4.2 Experiment 2: Ageing Rate of pork

The rate of ageing of pork loin was determined in experiment 2 and 50% of the improvements in tenderness for pork LTL occurred within two days post slaughter.

This is a similar ageing rate to that obtained by Dransfield *et al.* (1980-81) for pork LTL who used 1 cm x 1 cm cooked samples compressed in Volodkevich type jaws. The study by Dransfield and co-workers determined the rate constant to be 0.38, the same as that obtained in this experiment, with the relationship between toughness and storage time being: $WBSF = 5.3 + 4.4 e^{-0.38t}$, a similar relationship to that observed in this study. In Dransfield's study using twelve LTL muscles, the fitted curve accounted for 66% of the variation compared to 92.4% of the variation accounted for in this study using eight LTL muscles. Gould *et al.* (1965) reported similar high variability between pork carcasses (rate constant 0.47) to that seen by Dransfield *et al.* (1980-81). Measurements for beef and lamb have been shown to have a lower level of variability ($R^2 = 96\%$ for both, Dransfield *et al.*, 1980-81).

Variation in pH and temperature decline was observed to occur along the loin in experiment 2. The rate of pH decline was fastest at the 5th thoracic vertebra where the temperature decline was the slowest, probably explaining the differences seen in pH decline. The slower rate of temperature decline can be attributed to the greater amount of subcutaneous fat at this point relative to the other two sites assessed thus increasing the distance from the surface of the muscle thereby slowing the cooling rate. The rate of pH and temperature decline at the 12th thoracic vertebra and the 5th lumbar vertebra was very similar to each other. However, these rates of pH decline are slower than those reported in pork previously (Moller and Vestergaard, 1986; McFarlane and Unruh, 1996) but can probably be explained by the faster temperature decline than those previously reported (McFarlane and Unruh, 1996) as well as differences in genetics, pre-slaughter stress and chilling conditions between the different studies. The variation in pH seen along the LTL muscle was also reported in beef by Marsh *et al.* (1980-81) at 3 hours post slaughter. In the study by Marsh *et al.* (1980-81), a variation of more than 0.5 pH units was often found among samples taken from the middle third of a single muscle when tested using the iodoacetate-KCl method.

From experiment 2, it can be concluded that under the conditions of this experiment, 50% of tenderisation of pork LTL occurs within 2 days post slaughter and 80% within 4 days using the standardised conditions employed throughout. Thus to optimise pork tenderness for retail sale, a 4 day ageing period should be employed, longer than what is presently employed in the Australian pork market. This would obviously

result in higher storage costs but more tender pork. However this compares favourably to beef and lamb which require ageing periods of 10 days to maximise tenderness (Dransfield *et al.*, 1980-81). In subsequent experiments in this thesis, 4 days ageing was used as very few changes in tenderness were predicted to occur beyond this time. Furthermore, pH measurements were made either at the caudal end or at all three sites to ensure that the whole muscle was in rigor.

3.4.3 Experiment 3: Tenderisation of pork after accelerated boning

3.4.3.1 Cold Shortening

Accelerated boning combined with chilling at 0°C in experiment 3 resulted in considerable shortening of the LTL muscle with 15% muscle shortening, 21% muscle fibre shortening and a 25% reduction in sarcomere length compared with the conventionally boned muscles. This level of shortening is similar to that seen by Cross and Seidman (1985) and Iversen *et al.* (1994) who reported shortening of sarcomere lengths of 30 and 27%, respectively following accelerated boning.

In this study, muscles that underwent accelerated boning and chilling in iced water had shorter sarcomere lengths relative to the muscles which were conventionally boned supporting the original hypothesis of accelerated boning with chilling in iced water can result in cold shortening, a trend reported by others for pork (Swatland, 1995; Dransfield and Lockyer, 1985). The sarcomere length of the muscles conventionally boned were of similar length to those measured by Feldhusen and Kuhne (1992) in excised pork *longissimus dorsi* cooled at 5°C where the sarcomere length was 1.78 µm. Furthermore, muscles that underwent ultrarapid chilling in the study by Feldhusen and Kuhne (1992) had sarcomere lengths of 1.23 µm, which is similar to the sarcomere length of muscles which underwent accelerated boning in this experiment (1.34 µm) further suggesting cold shortening occurred in the muscles that underwent accelerated boning and chilling at 0°C in experiment 3 rather than shortening due to no skeletal restraint. In contrast to this experiment, Feldhusen and Kuhne (1992) observed a considerable lengthening of the sarcomeres from 3.75 hours post slaughter to 3 days post slaughter especially in the ultrarapid chilled muscles where the sarcomere length increased from 1.23 to 1.63 µm while little change in sarcomere length from 1 to 4 days post slaughter was observed in this

experiment. Furthermore, the differences in sarcomere length between the treatments in the study by Feldhusen and Kuhne (1992) disappeared by 3 days post slaughter.

The cold shortening observed in the muscles following accelerated boning and chilling at 0°C would have resulted from rapid temperature decline post slaughter and the removal of skeletal restraint. The fast temperature decline as a result of being placing in an iced water bath would be the main contributor to the shortening of the muscles which had undergone accelerated boning.

3.4.3.2 *Proteolytic activity*

The differences in tenderness observed between muscles following accelerated and conventional boning could also be seen in the changes in protein degradation patterns between 1 and 4 days post slaughter as indicated by the protein degradation patterns seen with SDS-PAGE. The muscles that had undergone accelerated boning did not appear to age to the same extent as the muscles that had undergone conventional boning as indicated by the changes to nebulin and troponin T and the formation of a 30 kDalton band for the muscles following conventional boning. The differences in protein bands between the treatments is in agreement to results reported in beef where differences in protein degradation have been observed between tough and tender muscles at 3 and 7 days post slaughter (Huff-Lonergan *et al.*, 1995; MacBride and Parrish, 1977). These results suggest that the increased toughness observed in the accelerated boned muscles is due to both cold shortening preventing ageing and the reduction in level of proteolytic activity supporting the original hypothesis that tenderness is reduced following accelerated boning due to cold toughening.

Muscles which had undergone accelerated boning had a lower myofibrillar fragmentation index relative to muscles which had been conventionally boned at both 1 and 4 days post slaughter further indicating a reduction in proteolytic activity in muscles which underwent accelerated boning. Similar MFI values were obtained to those previously reported for pork by Koohmaraie *et al.* (1991). These MFI results support the reduction in WBSF observed in muscles following accelerated boning. Increases in protein degradation were observed for both treatment groups as

indicated by an increased MFI from 1 day post slaughter to 4 days post slaughter. The increases in MFI between 1 and 4 days post slaughter seen in this experiment were not observed by Iversen *et al.* (1995) who observed no differences in MFI between samples taken at 1 and 7 days post slaughter.

Correlations between sarcomere length, MFI and WBSF were observed in experiment 3. An increase in sarcomere length was associated with a reduction in WBSF and increased MFI as would be expected. Moller and Jensen (1993) also observed a relationship between WBSF and sarcomere length for pork similar to that seen in this experiment. Feldhusen and Kuhne (1992) also reported a high correlation occurring between sarcomere length and WBSF of excised pork muscle after ultrarapid chilling.

In this study, the method of boning did not affect the total cathepsin B, B+L and D activities at 1 day post slaughter which contrasts with the MFI and SDS-PAGE results. Similar cathepsin activity values were obtained in this study to those previously reported in pork for cathepsin B and B+L (Koohmaraie and Kretchmar, 1990; Etherington *et al.*, 1987) and for cathepsin D (Afting and Becker, 1981; Caldwell and Grossjean, 1971). The lack of difference in total cathepsin activity between the treatments may be due to the time of measurement and the similarity in pH values at this time and due to the measurement of total activity rather than free and bound lysosomal activity.. Earlier collection may have resulted in differences between the treatments with cathepsin activity expected to be higher in the conventionally boned samples due to higher temperatures occurring at lower pH values, reducing the stability of the lysosomal membranes as shown by Moeller *et al.* (1976). In a study by Moeller *et al.* (1976) differences in total cathepsin activity were observed between muscles conditioned at different temperatures. The differences seen by Moeller *et al.* (1976) were seen during the early stages post slaughter but by 18 hours no differences were observed. This suggests that assessment at 1 day post slaughter may have been too late to determine if differences in their activities occurred. The earlier differences were observed by Moeller *et al.* (1976) due to differences in the rate of pH decline altering the stability of the lysosomal membranes. A faster rate of pH decline will result in an earlier release of the cathepsins from the lysosomes. However the ultimate activity is not altered.

3.4.3.3 Warner Bratzler peak shear force

WBSF was increased in muscles following accelerated boning relative to conventional boning at both 1 and 4 days post slaughter as originally hypothesised. The increased toughening in the muscles following accelerated boning can be attributed to both cold shortening and a reduction in proteolytic activity as indicated by the reduced MFI and reduced protein degradation. The increased toughness seen in the muscles following accelerated boning in experiment 3 has also been reported in beef and pork by other researchers. Woltersdorf and Troeger (1987) observed that accelerated boning of pork and chilling at 0°C resulted in increased toughness relative to conventional boning even after 4 days ageing. Moller and Jensen (1993) observed the increased toughness of excised pork *longissimus dorsi* muscle only occurred when the fast chilling rate in ice water was used whereas acceptable tenderness was obtained with chilling in air at 2-4°C. Feldhusen and Kuhne (1992) also reported reductions in tenderness associated with cold shortening after ultrarapid chilling of excised pork muscle but by 2 days post slaughter this reduction in tenderness relative to excised muscle chilled at 5°C had disappeared. The lack of difference in tenderness at 2 days post slaughter reported by Feldhusen and Kuhne (1992) can be explained by the massive increase in sarcomere length with ageing that they observed. The increase in sarcomere length with ageing can be explained by weakening of the Z discs during ageing and the disassociation of actin and myosin (Takahashi *et al.*, 1987; Hattori and Takahashi, 1988) thus improving tenderness.

Ageing for 4 days post slaughter improved the tenderness of the muscles which underwent both accelerated and conventional boning with similar WBSF values occurring at 1 and 4 days post slaughter to those seen by Warner *et al.* (1997). Although the cold shortening that was observed in muscles following accelerated boning resulted in tougher pork at 1 and 4 days post slaughter compared with muscles that had been conventionally boned, some ageing was observed as indicated by the reduction in WBSF, however no differences in the percentage change in WBSF from 1 to 4 days post slaughter was observed between the two treatments. Although the muscles which underwent accelerated boning showed some tenderness improvements, they still remained significantly tougher than the muscles which were conventionally boned (10.5 kg verses 5.6 respectively after 4 days ageing), and both treatments resulted in meat that would be unacceptable to the

consumer. A WBSF of less than 4.5 kg is considered to be acceptable to the consumer (Huffman *et al.*, 1996).

3.4.3.4 pH decline

The pH decline post slaughter of both treatments fitted an exponential curve. This pattern of pH decline for muscles following accelerated and conventional boning was similar to that observed for carbon dioxide stunned pigs by Iversen *et al.* (1994). Iversen *et al.* (1994) reported no difference in the rate of pH decline after boning the LTL at either 1 or 6 hours post slaughter and chilling in iced water or conventional boning and chilling at 2°C. However, Iversen *et al.* (1994) observed much faster rates of pH decline than those observed in this study. For example in their study the pH at 6 hours post slaughter for the muscles conventionally boned ranged from 5.65 to 5.9. This compares to a pH of 6.06 and 6.19 obtained in this experiment following conventional and accelerated boning respectively. These faster rates of pH decline reported by Iversen *et al.* (1994) may be due to different pre-slaughter handling stress and /or genetics with their pigs being Landrace x Duroc x Yorkshire as stunning, slaughter weight and chilling methods were similar in both studies.

No differences in muscle pH decline were observed between accelerated and conventional boning. The lack of difference between treatments in the rate of pH decline is in contrast to that seen in beef *semimembranous* by Follett *et al.* (1974). Follett *et al.* (1974) observed that conventionally boned muscles had a much faster rate of pH decline than the muscles which underwent accelerated boning and chilling at 5°C. The lack of difference in pH decline in our experiment between muscles that had undergone accelerated boning and those following conventional boning may be due to the highly efficient chilling system employed for the conventional boning. A fast temperature decline in the samples conventionally boned may have slowed the rate of pH decline resulting in similar values obtained in the muscles undergoing accelerated boning. Furthermore, the differences in pH decline with accelerated boning between the two studies may be explained by the anatomical differences between the muscles (*semimembranous* for beef, LTL for pork) influencing the temperature decline.

3.4.3.5 Colour, water holding capacity and protein denaturation

In this study accelerated boning resulted in a darker muscle (lower L*) colour at 1 day post slaughter relative to the muscles which underwent conventional boning. This effect has previously been reported in pork by van Laack (1989) and in beef by Shaw and Powell (1995). This colour difference has been attributed to a higher ultimate pH, faster cooling rate, reduced protein denaturation and a more densely packed myofibrillar protein matrix (that is shorter sarcomere lengths) in the muscles that had undergone accelerated boning (Shaw and Powell, 1995). However, in this experiment no differences were seen in ultimate pH or protein denaturation. Although no statistical differences in protein solubility were observed in this study, the small differences that occurred may have been sufficient to influence meat colour. The myofibrillar protein effect on surface lightness can be explained by the faster cooling resulting in reduced sarcomere length which would reduce the light scattering due to increased density of the myofibrillar proteins thereby giving the meat a darker appearance (Judge *et al.*, 1989).

As previously reported (Shaw and Powell, 1995; Iversen *et al.*, 1994), drip loss of pork loin muscle was increased by accelerated boning in experiment 3 relative to the conventionally boned muscle. This increased drip loss was also observed by Iversen *et al.* (1994) who reported much higher drip loss (15.08% for muscles boned 1 hour post slaughter compared to 12.67% for muscles which were conventionally boned) than what was reported in this experiment (4.78% for muscles which were accelerated boned and 3.02% for muscles which underwent conventional boning). The high drip loss observed by Iversen *et al.* (1994) can be explained by their use of frozen muscle samples relative to the use of fresh muscle samples in this experiment. The freezing process would result in damage to the myofibrillar structure thereby increasing the level of drip loss. The increased moisture loss in accelerated boned muscle seen in experiment 3 has also been reported in beef (Shaw and Powell, 1995). It is most likely that the increased drip loss was a result of the reduction in sarcomere length resulting in an increased compaction of the muscle proteins and thereby reducing the water holding capacity of the muscle as postulated by Marsh *et al.* (1972). This increase in drip loss can be attributed to the observation that most of the observed alteration in water holding capacity is due to the free water which is immobilised by the physical configuration of the muscles rather than due to the loss of bound water (van Laack, 1989).

Honikel (1986) showed that drip loss exhibits a close linear relationship to sarcomere length suggesting that drip loss can be attributed to the changes in the microstructure of the muscle and shrinkage of the myofibrils causing a greater proportion of free water to be lost (Marsh *et al.*, 1972). This linear relationship between drip loss and sarcomere length was not seen to occur in this experiment although a higher drip loss was seen with the reduction in sarcomere length of the accelerated boned muscles.

The increased drip loss seen in this study is in contrast to some other results previously published. Van Laack and Smulders (1992) did not find a difference in drip loss at 1, 5 or 12 days post slaughter between pork muscles which had undergone accelerated boning and conventional boning. This lack of change in drip loss found by van Laack and Smulders (1992) can be explained by the fact that no differences in sarcomere length were observed between the hot and cold boned which contrasts the results in experiment 3 where sarcomere length shortening was observed in muscles which under went accelerated boning.

The reduced water holding capacity of muscles that underwent accelerated boning was also observed in the purge and cooking loss measurements. An increase in purge at 4 days and in cooking loss at both 1 and 4 days post slaughter was observed in the muscles following accelerated boning relative to the muscles conventionally boned. This difference in cooking loss is in contrast to results by Iversen *et al.* (1994) in pork LTL who observed no differences in cooking loss between muscles following conventional boning and accelerated boning at either 1 or 6 hours post slaughter and chilled in ice water following boning.

Cooking loss was observed to increase in the aged samples relative to the fresh samples in experiment 3. Increased cooking loss in aged pork samples following both accelerated and conventional boning has also been reported by Warner *et al.* (1997). This increased cooking loss in experiment 3 between fresh and aged samples may have been due to the anatomical location of the samples. The aged samples were collected at the caudal end of the muscle which was shown in experiment 1 to have a higher cooking loss than the cranial end, the site of the fresh sample. Furthermore, cooking loss in aged samples would increase due to the weakening of the myofibrillar structure with ageing, thereby resulting in a reduced

water holding capacity of the muscle due to the fragmentation of the myofibrils releasing water.

The increased drip loss seen in this experiment was not associated with an increase in protein denaturation. Accelerated boned and conventionally boned muscles were observed to have similar protein solubility. This lack of difference in protein solubility suggests that the increased moisture loss was due to sarcomere length shortening rather than from protein denaturation. This lack of difference in protein solubility is in contrast to results found by van Laack and Smulders (1992) who reported less protein denaturation in accelerated boned pork and no differences in water holding capacity between the two treatments.

From experiment 3, it can be concluded that cold toughening of the muscles following accelerated boning resulted in a reduction in tenderness at 1 and 4 days post slaughter, the prevention of ageing, a darker meat colour and an increase in drip loss and reduction in water holding capacity relative to the conventionally boned muscles. Thus the original hypothesis that accelerated boning would result in cold toughening thereby reducing tenderness, surface lightness and increasing drip loss was supported.

3.5 CONCLUSIONS

From these experiments it was determined that tenderness did not vary along the loin muscle although there was an increase in cooking loss from the cranial to caudal end. By 4 days post slaughter 80% of tenderisation could be achieved in rigor boned muscle when stored at 2°C. Furthermore, it was seen that cold shortening and a reduction in proteolytic activity occurred in pork following accelerated boning and chilling at 0°C. The cold shortening caused an increased drip loss, produced a considerably darker surface colour, reduced proteolysis and decreased tenderness at 1 and 4 days post slaughter. Thus 4 days ageing may not have been sufficient to determine maximum improvements in tenderness and requires further investigation. Therefore, from these results accelerated boning combined with storage in iced water could not be recommended as an alternative to conventional boning and alterations to the boning method are required to produce a consumer acceptable product.

Figure 3.1. Anatomical description of division of *M. longissimus thoracis et lumborum* into 12 sections, 7 portions, 3 grouped portions and 2 halves for the determination of variation in Warner-Bratzler peak shear force at 1 day post slaughter.

	Cranial end					Caudal end	
dorsal surface	1	2	4	6	8	10	12
ventral surface		3	5	7	9	11	
	TV ^a 4	TV6	TV9	TV12	TV15	LV ^a 2	LV4
							LV6

^a TV = thoracic vertebra, LV = lumbar vertebra

Portions were grouped according to the following –

- a = section 1,
- b = sections 2 and 3,
- c = sections 4 and 5,
- d = sections 6 and 7,
- e = sections 8 and 9,
- f = sections 10 and 11 and
- g = section 12.

Grouped portions were grouped as follows –

- i = section 1,
- ii = sections 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11 and
- iii = section 12.

Halves were grouped as follows –

- A = sections 2, 4, 6, 8, and 10 and
- B = sections 3, 5, 7, 9 and 11.

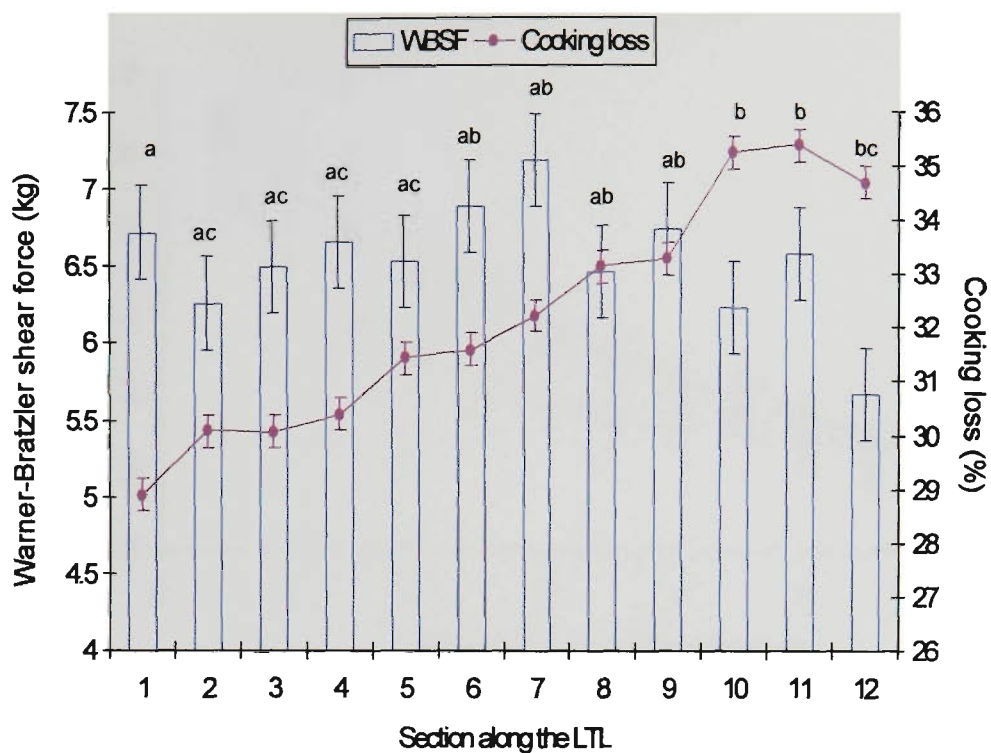


Figure 3.2 Variation in mean Warner-Bratzler peak shear force and cooking loss at 1 day post slaughter for the 12 sections of the *M. longissimus thoracis et lumborum* in experiment 1. Different letters denote a difference in cooking loss between sections. The vertical bars represent the standard errors.

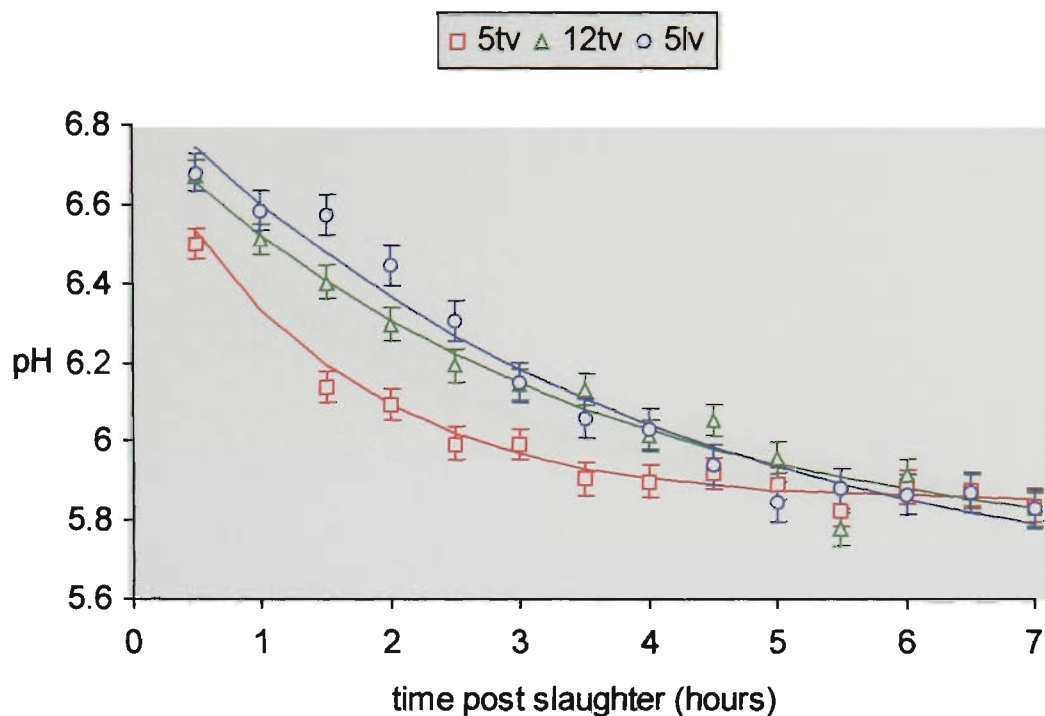


Figure 3.3 Rate of pH decline with time post slaughter in three sites of the *M. longissimus thoracis et lumborum* in experiment 2 from pork carcasses undergoing rigor boning. The sites were the 5th thoracic vertebrae (5tv), 12th thoracic vertebrae (12tv) and the 5th lumbar vertebrae (5lv). Each point represents the average pH for each time measured. The lines represent the fitted equation for each site. The vertical bars represent the standard errors.

The equations for the three sites are:

$$\text{pH}_{5\text{TV}} = 5.84 + 0.98e^{-0.69t} \quad (R^2 = 96.2, \text{RSD} = 0.04),$$

$$\text{pH}_{13\text{TV}} = 5.70 + 1.12e^{-0.31t} \quad (R^2 = 96.4, \text{RSD} = 0.05), \text{ and}$$

$$\text{pH}_{5\text{LV}} = 5.58 + 1.33e^{-0.27t} \quad (R^2 = 96.3, \text{RSD} = 0.06)$$

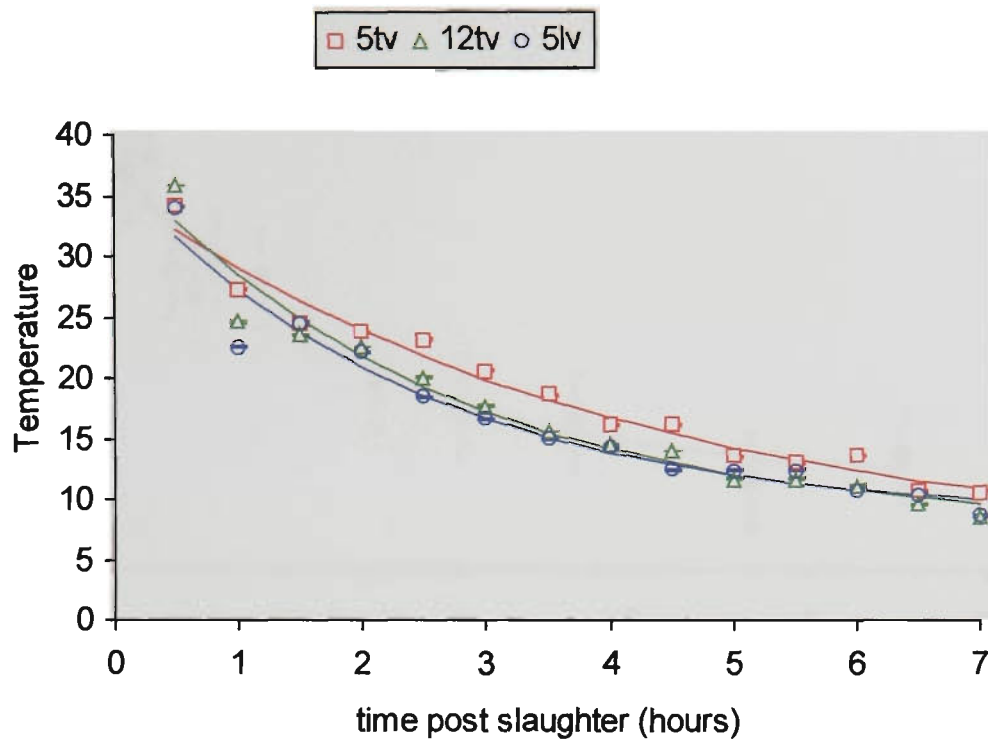


Figure 3.4 Average rate of temperature (°C) decline with time post slaughter along the *M. longissimus thoracis et lumborum* in experiment 2 from pork carcasses undergoing rigor boning. The three sites were the 5th thoracic vertebrae (5tv), 12th thoracic vertebrae (12tv) and the 5th lumbar vertebrae (5lv). Each point represents the average temperature for each time measured. The lines represent the fitted equation for each site. The vertical bars represent the standard errors.

The equations for the three sites are:

$$\begin{aligned} \text{temp}_{5\text{TV}} &= 5.59 + 30.20e^{-0.25t} \quad (R^2 = 96.8, \text{RSD} = 1.23), \\ \text{temp}_{13\text{TV}} &= 7.68 + 30.75e^{-0.39t} \quad (R^2 = 95.3, \text{RSD} = 1.63) \text{ and} \\ \text{temp}_{5\text{LV}} &= 8.42 + 28.53e^{-0.414t} \quad (R^2 = 93.7, \text{RSD} = 1.75) \text{ where time is in hours.} \end{aligned}$$

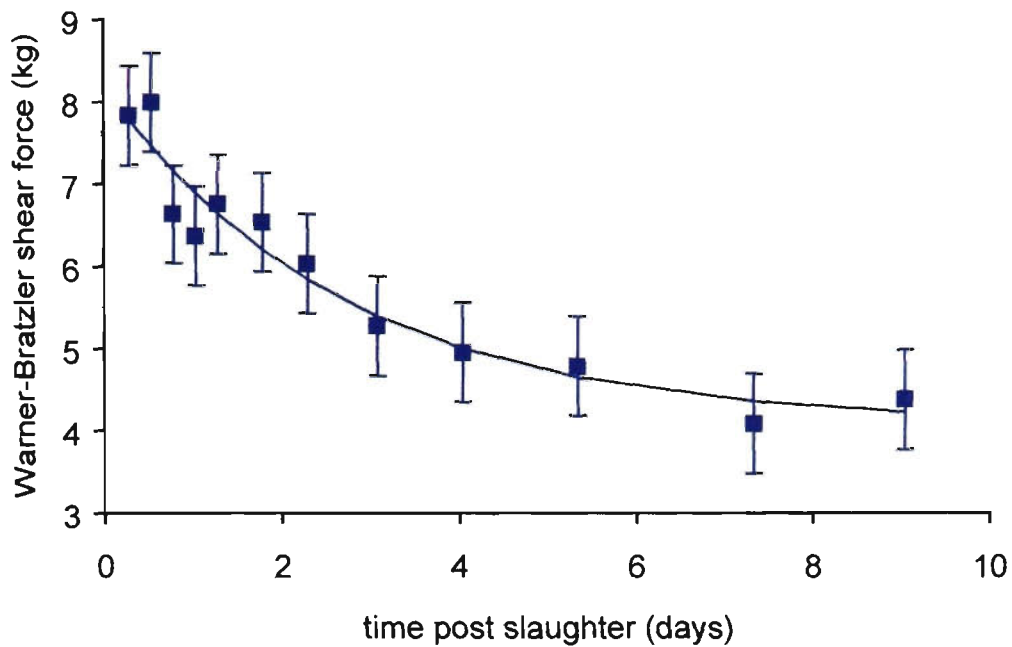


Figure 3.5 Changes in Warner-Bratzler shear force of pork *M. longissimus thoracis et lumborum* in experiment 2 over a 10 day period after undergoing rigor boning. Each point represents the average WBSF for each time measured. The line represents the fitted equation for change in WBSF with time post slaughter. The vertical bars represent the standard errors. The equation is $WBSF = 4.08 + 4.14 e^{-0.38time}$ where time is measure in days post slaughter.

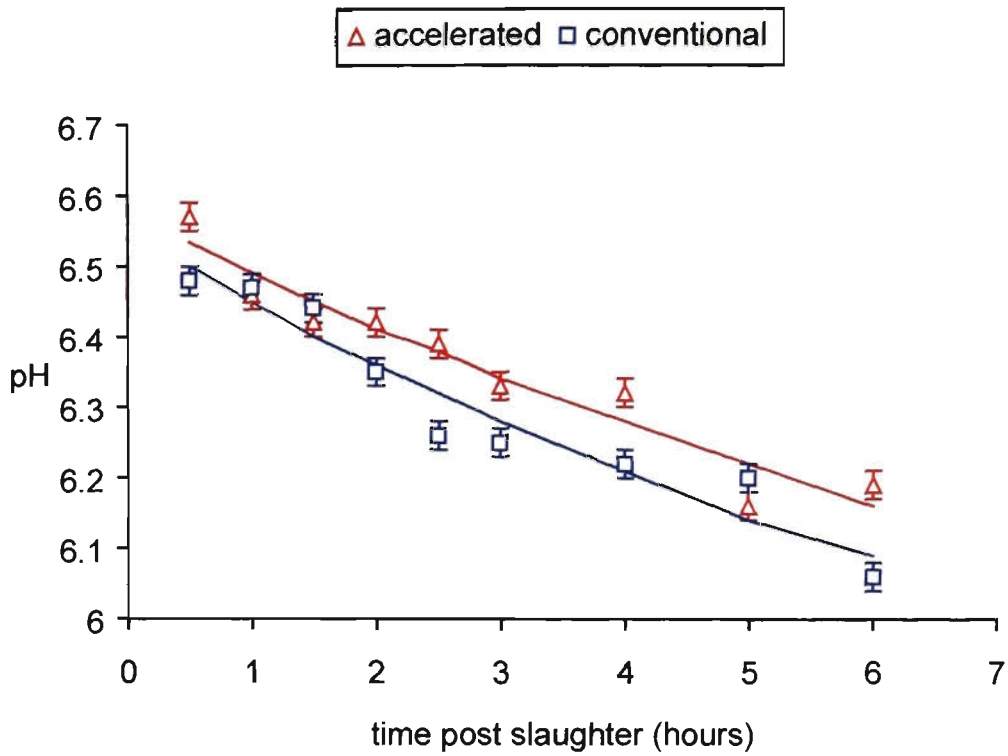


Figure 3.6 The average rate of pH decline of pork *M. longissimus thoracis et lumborum* in experiment 3 after accelerated and conventional boning. Each point represents the average pH for each time measured. The lines represent the fitted equation for each site. The vertical bars represent the standard errors.

The equations are:

$$pH_{CB} = 5.66 + 0.89e^{-0.12t} \quad (R^2 = 97.2, \text{RSD} = 0.04) \text{ and}$$

$$pH_{AB} = 5.67 + 0.91e^{-0.10t} \quad (R^2 = 97.4, \text{RSD} = 0.04).$$

Figure 3.7 Representative SDS-PAGE showing the difference in protein bands following ageing of pork *M. longissimus thoracis et lumborum* myofibrils after accelerated (AB) and conventional (CB) boning. Sample lanes were loaded with 10 µl of 4 mg/ml total protein and the gel was 10% acrylamide (w/v), 0.06% bis-acrylamide (w/v), pH 9.3. Protein bands identified are; N = nebulin M = myosin, A = actin, 30kD = 30 kDalton protein. The lanes from left to right are – molecular weight standards (molecular weights (kDalton) are indicated), AB 1 day, AB 4 days, CB 1 day and CB 4 days post slaughter.

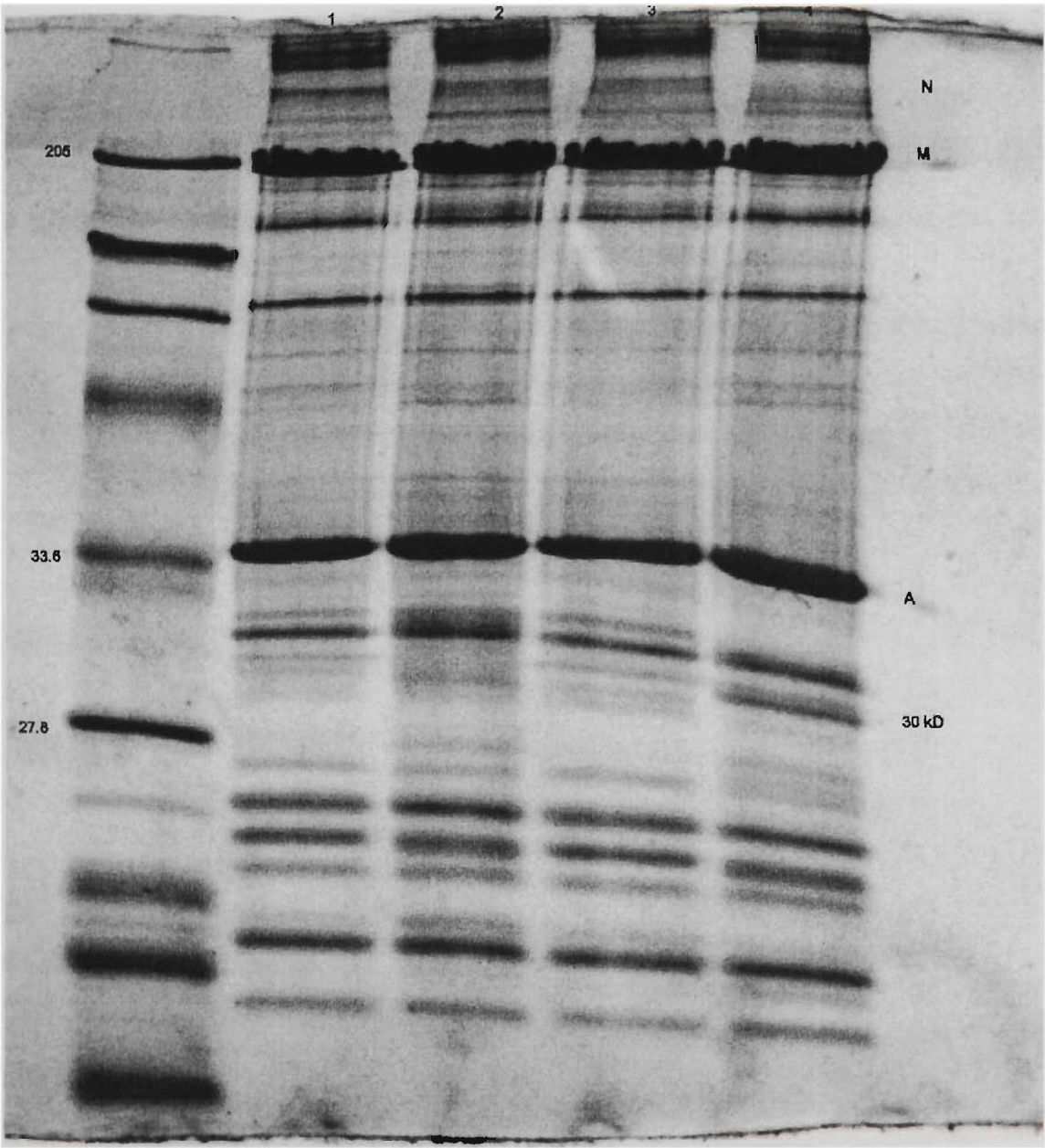


Table 3.1 Meat quality traits of pork *M. longissimus thoracis* at 1 day post slaughter after accelerated and conventional boning. The values are least squares means and SED = standard error of the difference.

	Processing		Significance ¹	SED
	Conventional	Accelerated		
pH	5.71	5.75	NS	0.059
drip loss (%)	3.02 ² (1.10) ^a	4.78 ² (1.57) ^b	P<0.05	0.164
L*	48.8 ^a	44.1 ^b	P<0.01	1.03
a*	4.43 ^a	3.99 ^b	P<0.05	0.163
b*	2.95 ^a	2.10 ^b	P<0.05	0.267

^{ab} within rows, means with different superscript differ significantly (P<0.05),

¹NS = no significant differences.

²Drip loss means before log transformation, transformation means in brackets below.

Table 3.2 Warner-Bratzler peak shear force, sarcomere length and myofibrillar fragmentation index at 1 day and 4 days post slaughter for pork *M. longissimus thoracis* after accelerated and conventional boning. The values are least squares means and SED = standard error of the difference.

	Processing		Significance	SED
	Conventional	Accelerated		
WBSF ¹ 1 day post slaughter	7.0 ^a	12.7 ^b	P<0.01	1.16
WBSF 4 days post slaughter	5.6 ^a	10.5 ^b	P<0.001	0.52
% change WBSF ²	19.8	13.9	P > 0.05	10.62
S L ³ 1 day post slaughter	1.78 ^a	1.34 ^b	P<0.001	0.053
S L 4 days post slaughter	1.77 ^a	1.36 ^b	P<0.001	0.041
MFI ⁴ 1 day post slaughter	61.4 ^a	39.0 ^b	P<0.05	7.41
MFI 4 days post slaughter	102.1 ^a	63.4 ^b	P<0.05	13.53

^{ab} within rows, means with different superscript differ significantly (P<0.05),

¹WBSF = Warner-Bratzler peak shear force (kg).

²% change WBSF = percentage change in WBSF from 1 to 4 days post slaughter

³S L = sarcomere length (μm)

⁴MFI = myofibrillar fragmentation index

Table 3.3 Total, sarcoplasmic and myofibrillar protein solubility (mg/g) and total cathepsin B, B+L and D activity of pork *M. longissimus thoracis et lumborum* at 1 day post slaughter after accelerated and conventional boning. The values are least squares means and SED = standard error of the difference.

	Processing		Significance ¹	SED
	Conventional	accelerated		
Total protein solubility	88.3	103.8	NS	10.48
Sarcoplasmic protein solubility	44.1	39.1	NS	2.33
Myofibrillar protein solubility	44.2	64.7	NS	11.05
Cathepsin B ²	2.70	2.87	NS	0.176
Cathepsin B+L ²	8.55	9.60	NS	1.933
Cathepsin D ³	3.28	3.36	NS	0.138

¹NS = no significant differences.

²Cathepsin B and B+L activity = 1 unit of enzyme releases 1 µmol of product per minute.

³Cathepsin D 1 unit of specific activity = an increase of 0.1 absorbance units at 750 nm per 1 hour of incubation at 37°C.

Table 3.4 Cooking loss and purge measured at 1 day and 4 days post slaughter of pork *M. longissimus thoracis et lumborum* after accelerated and conventional boning. The values are least squares means and SED = standard error of the difference.

	Processing		Significance	SED
	Conventional	Accelerated		
purge (%)	3.0 ^a	4.5 ^b	P<0.01	0.40
cooking loss (%) fresh ¹	29.1 ^a	34.0 ^b	P<0.001	0.96
cooking loss (%) aged ²	32.6 ^a	35.0 ^b	P<0.001	0.34

^{ab} within rows, means with different superscripts differ (P<0.05),

¹Fresh = 1 day post slaughter, ²aged = 4 days post slaughter.

4. IMPROVING TENDERNESS AFTER ACCELERATED BONING WITH TEMPERATURE CONDITIONING, POST SLAUGHTER AGEING AND CALCIUM CHLORIDE.

4.1 INTRODUCTION

In chapter 3, accelerated boning of pork loin followed by chilling at 0°C, resulted in tougher pork and higher drip loss due to cold toughening. Thus, alterations to the accelerated boning methods used for pork are required to overcome the problem of cold shortening and reduced proteolytic activity. Pearson (1986) has shown that the increased toughness associated with cold toughening can be overcome by temperature conditioning while Koohmaraie *et al.* (1988) has shown post slaughter ageing and the infusion of calcium chloride can also be used to overcome increased toughness. The experiments in this chapter were designed to determine the optimal method to maximise tenderness and improve overall pork quality following accelerated boning, and to examine the rate of ageing of accelerated boned pork.

4.1.1 Temperature conditioning of pork following accelerated boning

Temperature conditioning, the chilling of muscles at elevated temperatures to slow the rate of temperature decline, is believed to be one method that can be used to improve tenderness following accelerated boning. The main impact muscle temperature has is on the rates of both glycolytic and proteolytic reactions and on the extent of muscle shortening.

The rates of pH and of temperature decline during rigor development are believed to be two of the most important factors controlling tenderisation and ageing of pork. The rate of chilling of the muscle during rigor development influences both glycolytic and proteolytic reaction rates, thereby influencing the rate of pH decline (Marsh *et al.*, 1987; Pike *et al.*, 1993) and the rate of tenderisation (Wang *et al.*, 1992; Moeller *et al.*, 1977, 1976). Variation in the rate of rigor development have been reported to alter the muscle structure, the release of calcium ions and the activity of calpains by up to 100-

fold (Dransfield, 1994). The activity of μ -calpain decreases very rapidly when the pH of beef muscle drops sharply (Wang *et al.*, 1992). Thus the post-mortem rate of pH decline of muscle might be the main factor affecting the calpain activity and stability (Wang *et al.*, 1992). This suggests that temperature conditioning may also effect the activities of major tenderising enzymes due to the effects of temperature decline on pH decline post slaughter.

Temperature conditioning has also been shown to increase cathepsin activity due to earlier release of the enzymes from the lysosomes while temperatures are still high (Moeller *et al.*, 1977; Moeller *et al.*, 1976). A low muscle pH combined with high carcass temperature post slaughter enhances the disruption of the lysosomal membranes, releasing lysosomal enzymes into the muscle tissue (Moeller *et al.*, 1976; Moeller *et al.*, 1977, Dutson *et al.*, 1977). Holding the carcass at elevated temperatures not only stimulates the release of lysosomal enzymes from the lysosomes but also increases the activity of these enzymes during the early hours post slaughter (Moeller *et al.*, 1976). This increased activity is due to the combined effect of high temperature and low pH which approaches the optimal conditions for these enzymes (Zeece and Katoh, 1989).

Cathepsin-C activity has been shown to be altered by temperature conditioning (Moeller *et al.*, 1976; Moeller *et al.*, 1977). Cathepsin-C activity released from the lysosomes at 12 hours post mortem is increased in beef *longissimus dorsi* muscle with high temperature conditioning at 22°C for 4 hours post mortem, 12°C for an additional 8 hours and chilling to 2°C compared to muscles immediately chilled at 2°C (Moeller *et al.*, 1976). These differences in enzyme activity disappeared by 18 hours post mortem, indicating differences in the activities of enzymes and the release of the enzymes from the lysosomes occurred early post mortem. Thus, the same degree of lysosomal enzyme release is finally reached in the control sides at 18 hours post slaughter (Moeller *et al.*, 1976) and the differences in tenderness that result due to temperature conditioning can be explained by earlier activation of tenderising enzymes in the temperature conditioned muscles.

However, care must be taken to ensure that the appropriate temperature is used to condition muscles following accelerated boning. If the temperature is too low, the

muscle will cold toughen (Pearson and Young, 1989). Cold toughening involves both cold shortening and the reduced activity of the proteolytic enzymes due to the cold temperature (Dutson and Pearson, 1985). Previously reported chilling conditions for normal pork muscle to prevent cold shortening are to chill to temperatures below 20°C within four hours post mortem and not to chill below 10°C before five hours post slaughter (Honikel and Reagan, 1987). This enables the onset of rigor mortis at a pH of around 6.0 to occur at 15-18°C (Honikel and Reagan, 1987). Under these conditions of rigor mortis occurring at 15-18°C, there is a minimum shortening of sarcomeres, resulting in meat with low drip loss and high tenderness (Locker and Hagyard, 1963). However if the temperature is too high, a rapid rate of muscle pH decline may be induced. This could result in heat shortening, protein denaturation, reduced water holding capacity and a paler meat colour producing PSE pork.

Temperature conditioning improves meat tenderness following accelerated boning, however conflicting results are presented on the optimal temperature and length of time at which muscles should be conditioned. For example, Pearson (1986) found storing pre rigor beef *longissimus dorsi* following accelerated boning at temperatures around 16°C until the onset of rigor mortis prevented cold shortening. This is probably due to the effect of higher temperatures on slowing the release of calcium ions and increasing the capacity of the sarcoplasmic reticulum to take up excess calcium, thus reducing contraction and enabling the muscles to relax (Pearson, 1986). Similarly, Schmidt and Gilbert (1970) found that the potential cold toughening effects of accelerated boning on beef *biceps femoris* and *longissimus dorsi* tenderness could be overcome by temperature conditioning at 15°C for 24 or 48 hours. Schmidt and Gilbert (1970) also reported that the muscles held at 15°C for 24 hours were as tender as the controls (chilled at 9°C on the carcass) and those held for 48 hours were more tender than the controls. However, Schmidt and Keman (1974) found that the impact of accelerated boning on beef tenderness could be overcome by chilling beef *longissimus* muscle removed from the carcass within 1 hour post slaughter at 7°C for 4 hours followed by ageing at 1°C for 8 days. Furthermore, Follett *et al.* (1974) reported temperature conditioning of excised beef *semimembranosus* at 5, 10 and 15°C for 24 hours followed by ageing at 1°C for 3, 7 or 13 days post slaughter ensured tenderness. In contrast, Butcher (1977) found temperature conditioning at 5-10°C for 24 hours reduced tenderness of accelerated boned beef. Thus a wide range of temperatures have been

reported to be effective at overcoming the negative impact on tenderness of accelerated boning for beef ranging from as low as 5°C to as high as 16°C.

Similar to the results for beef, conflicting results have been observed for the optimal temperature at which pork should be chilled following accelerated boning. Honikel and Reagan (1987) observed cold shortening of pork in muscles held at 5°C in a water bath until 5 hours post slaughter relative to muscles held at 11 or 17°C following accelerated boning and compared to muscles chilled on the carcass at 3°C. In contrast Dransfield and Lockyer (1985) observed that excised pork loins held at 20°C tended to be tougher than those held at 5°C.

The response of tenderness to the conditioning temperature can be influenced by the rate of pH decline. Moller and Vestergaard (1988) reported that conditioning pork loin muscle at 15°C for 5 hours when the pH at one hour post slaughter was between 6.1 and 6.5 resulted in higher Warner-Bratzler peak shear force (WBSF) values than muscles which had been conventionally boned and muscles which had undergone accelerated boning and temperature conditioned at 30°C for either 60 or 90 minutes. However, Moller and Vestergaard (1988) reported when the pH was between 5.7 and 6.1 at one hour post slaughter, muscles which had undergone accelerated boning and temperature conditioned at 15°C were as tender as the muscles which had been conventionally boned and muscles which had undergone accelerated boning and temperature conditioned at 30°C for either 60 or 90 minutes. The improvements in tenderness can be explained by proteolysis due to higher temperatures occurring in the muscles when rigor onset occurs.

To maintain tenderness of pork following accelerated boning, chilling rates similar to that seen with conventional boning should be maintained. In Germany, pig sides chilled conventionally, have shown LTL muscle temperatures around 25°C at 3 hours post slaughter with a pH of 6.2 and 18°C at 5 hours post slaughter when the pH is around 6.0 (Honikel, 1986). LTL muscles following conventional boning and chilling at 2°C in experiment 2 resulted in a pH of 6.1 and temperature of 18°C at 3 hours post slaughter and a pH of 5.96 and temperature of 12°C at 5 hours post slaughter. In order to maintain acceptable tenderness, the temperatures used for chilling pork following accelerated boning should result in similar temperatures at 3 to 5 hours post slaughter

to those observed in muscles following conventional boning. Temperature conditioning muscles at 21°C following accelerated boning should result in similar muscle temperature at rigor as would occur in pork that had been conventionally boned.

Thus experiment 4 was designed to test the following hypotheses;

- 0°C will decrease tenderness due to cold shortening and a reduction in proteolytic activity
- 7°C will decrease tenderness due to reduction in proteolytic activity without cold shortening
- 14°C will result in tender pork by preventing cold toughening without a negative impact on meat quality. This treatment will confirm earlier reports that a muscle temperature of 14-15°C is required when the pH falls below 6.0 to prevent cold shortening
- 21°C will improve tenderness by preventing cold toughening but may possibly have a negative impact on meat quality due to possible protein denaturation. Furthermore 21°C was used to mimic the muscle temperature that would occur in conventionally boned muscles at rigor.

4.1.2 Rate of ageing of pork following accelerated boning

Another method reported to improve meat tenderness is post slaughter ageing. However conflicting reports are presented on the ideal storage time. Furthermore, conflicting evidence exists on whether post slaughter storage of cold shortened muscle is effective in improving tenderness.

Meat becomes more tender during storage post rigor (Dransfield *et al.*, 1980-81), a process referred to as ageing. Tenderisation is believed to occur because of the degradation of some of the key structural proteins (desmin, titin and nebulin) by endogenous enzymes when meat is aged (Koohmaraie *et al.*, 1995). Furthermore, changes in the sarcoplasmic proteins, actomyosin and connective tissue have been observed during ageing along with myofibrillar fragmentation that occurs in or near the z disc (Bandman and Zdaris, 1988; Koohmaraie, 1994). This fragmentation has been related to increased tenderness (Bandman and Zdaris, 1988; Koohmaraie, 1994).

There are differing views on the optimum time of post mortem ageing to achieve pork tenderness following conventional boning. Harrison *et al.* (1970) and Buchter and Zeuthen (1971) observed an increase in tenderness in pork LTL muscle up to the sixth and eighth days post mortem respectively. In contrast, Bennett *et al.* (1973) found that ageing more than one to two days did not significantly improve the tenderness of pork. Furthermore, Feldhusen and Kuhne (1992) found that the optimum shear force values for pork were attained after two to three days of ageing. Dransfield *et al.* (1980-81) used a model to predict that on average 50% of the tenderisation of pork occurs in two days relative to 4.2 days for beef and veal under their experimental conditions.

In a previous experiment (chapter 3), the ageing rate of Australian pork using standardised conditions (standard genetics, transport, lairage, handling, carbon dioxide stunning and chilling) and boning post rigor ($\text{pH} < 5.8$) was studied. The relationship between average WBSF and the time of storage was determined and the equation predicted that 50% of tenderisation had occurred by 2 days post slaughter and 80% of ageing had occurred by 4 days post slaughter.

Furthermore, conflicting views are presented in the literature on the degree of ageing of meat following accelerated boning. The lack of improvement in tenderness with post slaughter storage of muscle following accelerated boning is believed to be due to cold toughening. Davey *et al.* (1967) demonstrated that with increased muscle shortening the muscle demonstrates a decrease in tenderness improvements with ageing. The reduced tenderness improvement when muscles shorten is believed to be caused by structural changes in the myofibrils preventing the proteolysis by endogenous enzymes of proteins on or near the z line (Iversen *et al.*, 1995). Although in chapter 3 it was shown that the tenderness of the muscle following accelerated boning was still tougher than the conventionally boned muscle at 4 days post slaughter, some improvement in tenderness was observed. Thus it is possible that the tougher muscle resulting from accelerated boning may require longer to reach its optimal tenderness. Therefore a closer examination of the rate of ageing is required.

Thus experiment 5 was designed to test the following hypotheses;

- muscles undergoing accelerated boning and chilling at 0°C will not age due to cold shortening and a reduction in proteolytic activity

- muscles undergoing accelerated boning and chilling at 14°C will tenderise to acceptable levels within 6 days post slaughter due to the prevention of cold shortening and maximisation of proteolysis
- muscles undergoing rigor boning will undergo 80% of the improvements in tenderness within 4 days post slaughter as previously observed in chapter 3.

4.1.3 Effect of calcium chloride infusion on pork tenderness

A third method reported to improve tenderness of muscle is the infusion of calcium chloride post slaughter. The majority of studies on calcium chloride infusion have been conducted in beef and lamb. Significant improvements in beef tenderness have been observed after calcium chloride infusion (Morgan *et al.*, 1991; Wheeler *et al.*, 1991; Geesink, 1993; Boleman *et al.*, 1995). Several studies have shown that infusion of 0.3 M calcium chloride into pre-rigor bovine muscle has resulted in almost complete tenderisation within one day post mortem (Morgan *et al.*, 1991; Wheeler *et al.*, 1991 and Geesink, 1993). Furthermore calcium chloride treated muscles are more tender than non-treated muscles after ageing (Morgan *et al.*, 1991; Wheeler *et al.*, 1991 and Geesink, 1993). Studies of calcium chloride infusion into post rigor beef muscle have also reported improvements in tenderness (Boleman *et al.*, 1995, Wheeler *et al.*, 1993). Boleman *et al.* (1995) showed that infusion of beef *semimembranosus* at 60 minutes post slaughter was the most effective time to result in reduced shear force values and prevent excess moisture loss. However, infusion at 12 or 24 hours post slaughter was also effective in lowering shear force values relative to non-infused muscles (Boleman *et al.*, 1995).

Improvements in beef tenderness have also been observed after the addition of other chloride salts. Alarcon-Royo and Dransfield (1995) observed infusion of post rigor beef muscle with various salt solutions showed sodium and potassium salts were 43% and magnesium salts 73% as effective as calcium salts in tenderising *semitendinosus* muscle. These results support the hypothesis by Wu and Smith (1987) that the mechanism for tenderness improvements is due to a change in the ionic strength. The change in ionic strength due to infusion is thought to result in a weakening in the structural integrity of the myofibrils. Thus it is probable that activation of calpains alone

is not responsible for the observed change in tenderness of muscles following calcium chloride infusion.

Calcium chloride infusion of lamb muscles have also resulted in improved tenderness (Koohmaraie and Shackelford, 1991; Koohmaraie, 1990; Koohmaraie *et al.*, 1990; Koohmaraie *et al.*, 1989; Koohmaraie *et al.*, 1988b). Koohmaraie *et al.* (1988b) reported the infusion of lamb carcasses at slaughter with 0.3 M calcium chloride along with electrical stimulation resulted in lower shear force values at 24 hours. This was compared to samples that received only electrical stimulation, those that received electrical stimulation and water infusion and those that received no calcium chloride infusion and no electrical stimulation. The lower shear force values did not change with further storage relative to carcasses that were not electrically stimulated or infused with calcium chloride. Thus these results suggest that calcium chloride eliminates the requirement for ageing of lamb. At 24 hours post slaughter, muscles undergoing calcium chloride and electrical stimulation treatments were more tender than those that were only electrically stimulated. However by 6 days post slaughter, no differences in tenderness were seen between the muscles which were only electrically stimulated and the muscles which received both electrical stimulation and calcium chloride infusion (Koohmaraie *et al.*, 1988b). Thus calcium chloride reduces the need for post slaughter ageing.

Three different mechanisms have been proposed as the means by which calcium chloride improves tenderness but its effect is probably due to a combination of these mechanisms. Calcium chloride is thought to improve tenderness via increasing the activation of the calpains (Koohmaraie *et al.*, 1988; Koohmaraie *et al.*, 1989), via causing extreme contraction of the muscle fibres resulting in disruption of the myofibrillar network (Morgan *et al.*, 1991) or via altering the protein to protein interactions due to the elevated ionic strength (Wu and Smith, 1987). Although calcium chloride infusion has been shown to increase the activity of calpain enzymes (Koohmaraie *et al.*, 1988; Koohmaraie *et al.*, 1989), little is known on the influence of ion infusion on the release of cathepsins from the lysosomes. The change in ionic strength may also influence the stability of the lysosomal membranes, the release of the cathepsins and contribute to the improved tenderness seen in ion infused muscle.

The effect of calcium chloride infusion on pork tenderness and other meat quality attributes is not well known, thus experiment 6 was designed to test the following hypothesis;

- calcium chloride infusion will improve tenderness of pork and reduce the need for post slaughter ageing due to activation of the proteolytic enzymes, increased ionic strength and increase severe muscle contraction regardless of processing method
- calcium chloride infusion will prevent cold shortening and accelerate proteolysis by altering the glycolytic rate. However calcium chloride may cause massive muscle contraction, damaging the myofibrillar structure and thereby improving tenderness.
- infusion of calcium chloride at 0.5 hours post slaughter will result in greater improvements in tenderness than infusion at 6 hours due to earlier changes in ionic strength and earlier activation of the proteolytic enzymes
- temperature conditioning following accelerated boning is not required to improve tenderness when calcium chloride infusion is applied.

4.2 METHODOLOGY

4.2.1 Experiment 4: Temperature conditioning of pork following accelerated boning

Eight Landrace x Large White female pigs were slaughtered on one day, split in half and the *M. longissimus thoracis et lumborum* (LTL) removed from both sides and trimmed of subcutaneous fat within 0.5 hours post mortem. The LTL from each side was cut in half at the 12th thoracic vertebrae and the halves randomly assigned to a temperature conditioning treatment after packaging in sealed plastic bags. The conditioning treatments consisted of:

- AB-0 - chilling in a 0°C iced water bath,
- AB-7 - chilling in a 7°C water bath,
- AB-14 - chilling in a 14°C water bath or,
- AB-21 - chilling in a 21°C water bath.

Temperature conditioning was performed in temperature controlled water baths until 60 minutes after pH < 5.8 was reached (rigor) or until the pH failed to fall over a 2 hour period. The muscles were then cut into two – one (dorsal end) for immediate

assessment of meat quality and the other (ventral end) for ageing for 4 days at 2°C after vacuum packaging.

4.2.1.1 *Meat quality measurements*

Temperature and pH of the LTL muscle was measured every 0.5 hours for the first 3 hours post slaughter then every hour until rigor as defined previously. At both rigor and 4 days post slaughter, the muscles were assessed for pH, instrumental colour (CIE-L*, a*, b*), cooking loss and Warner-Bratzler peak shear force (WBSF) using fresh samples (that is, not frozen). In addition at rigor, samples were removed for measurement of drip loss and at 4 days post slaughter the purge was measured. All methods used are described in chapter two.

At rigor and 4 days post slaughter, a 50 g sample was frozen in liquid nitrogen and stored at -80°C for six weeks for biochemical measurements.

4.2.1.2 *Biochemical measurements*

At rigor, a fresh muscle sample was used to determine total cathepsins B, B + L and D activity. The samples previously frozen at -80°C were processed for the measurement of myofibrillar fragmentation index (MFI), sarcomere length, and separation of myofibrillar proteins on SDS-PAGE. All methods used are outlined in chapter two.

4.2.2 Experiment 5: Rate of ageing of pork following accelerated boning

Fifteen Large White x Landrace female pigs were slaughtered on three separate days and the sides were randomly allocated to one of three treatments:

- (i) RB – rigor boning, placed in the chiller and boned at rigor ,
- (ii) AB-0 – accelerated boning within 0.5 hours of slaughter and placed in ice water bath (0°C) until rigor, and
- (iii) AB-14 – accelerated boning within 0.5 hours of slaughter and temperature conditioned at 14°C in a water bath until rigor.

Rigor was defined as 1 hour after pH < 5.8 was reached or the pH did not change over a two hour period. At rigor, the LTL muscle was cut into twelve approximately 150± 10

gram samples, randomly allocated to an ageing period, vacuum packaged and stored at 2°C. The ageing periods were 0.75, 1, 1.25, 2, 3, 4, 6, 8 and 10 days post slaughter.

4.2.2.1 *Meat quality measurements*

The pH of the LTL was monitored every 60 minutes to determine rigor. At rigor, 0.75, 1, 1.25, 2, 3, 4, 6, 8, and 10 days post slaughter the WBSF and cooking loss of the muscle was measured. Purge levels were measured at 4, 6, 8 and 10 days post slaughter. Instrumental colour and pH were measured at rigor and 4 days post slaughter and samples were removed for measurement of drip loss at rigor. Samples (50 ±10 g) for biochemical measurements were collected at rigor and again at 4 days post slaughter, frozen in liquid nitrogen and stored at -80°C for assessment within 6 weeks. All methods used are outlined in chapter two.

4.2.2.2 *Biochemical measurements*

Samples collected and frozen at rigor and 4 days post slaughter were assessed for myofibrillar fragmentation index, sarcomere length and separation of myofibrillar proteins on SDS-PAGE using the methods outlined in chapter two.

4.2.3 Experiment 6: Effect of calcium chloride infusion on pork tenderness

Thirty-six Landrace x Large White female pigs were slaughtered over three days and randomly allocated to one of 9 treatments using a factorial combination of processing x infusion x time. The processing treatments were rigor boning (RB), accelerated boning followed by chilling at 0°C in an iced water bath (AB-0) and accelerated boning followed by chilling at 14°C in a water bath (AB-14). The infusion treatments were no infusion, infusion with calcium chloride infusion at 0.5 hours post slaughter (Ca0.5) and infusion with calcium chloride infusion at 6 hours post slaughter (Ca6). All treatments were applied to the LTL. Thus the treatments were:

- (i) RB : rigor boning with no ion infusion,
- (ii) RB-Ca0.5 : rigor boning, with calcium chloride infusion into the LTL muscle on the carcass at 0.5 hours post slaughter,

- (iii) RB-Ca6 : rigor boning with calcium chloride infusion 6 hours post slaughter,
- (iv) AB-0 : accelerated boning, placed in an ice water bath (0°C) with no ion infusion,
- (v) AB-0-Ca0.5 : accelerated boning, placed in an iced water bath (0°C), with calcium chloride infusion 0.5 hours post slaughter into boned LTL,
- (vi) AB-0-Ca6 : accelerated boning, placed in an ice water bath (0°C), with calcium chloride infusion 6 hours post slaughter into boned LTL,
- (vii) AB-14 : accelerated boning, place in a 14°C water bath, with no ion infusion,
- (viii) AB-14-Ca0.5 : accelerated boning, placed in a 14°C water bath, with calcium chloride infusion 0.5 hours post slaughter into boned LTL,
- (ix) AB-14-Ca6 : accelerated boning, placed in a 14°C water bath, with calcium chloride infusion 6 hours post slaughter into boned LTL.

Accelerated boned samples were boned and trimmed of fat and connective tissue at 0.5 hours post slaughter, and the rigor boned samples were removed at rigor, as previously defined.

Calcium chloride was infused at a concentration of 0.3 M to 10% (w/w) of the original weight using a hand held brine injector fitted with a three prong infusion fork. For the rigor boned muscles, the pump level was determined by the weight of the accelerated boned LTL muscle. The calcium chloride was made up with deionised, distilled water and infused at 30°C at 0.5 hours post slaughter, or at the temperature of the muscle at 6 hours post slaughter (14°C for accelerated boned muscles temperature conditioned at 14°C and the samples still on the carcass and 0-2°C for the accelerated boned LTL held at 0°C). The change in weight following infusion of calcium chloride of the LTL was determined for the accelerated boned samples.

4.2.3.1 *Meat quality measurements*

The pH and temperature of the LTL at the 5th and 6th lumbar vertebrae was measured every 60 minutes post slaughter until rigor mortis had set in. At rigor, the muscles were assessed at the 12th thoracic vertebrae for colour (Minolta CIE-L*, a*, b*), temperature, drip loss, WBSF and cook loss. The remaining LTL was cut into four approximately 150 ± 10 gram samples, vacuum packaged, randomly allocated to ageing periods of 1,

2, 4 and 6 days post slaughter and stored at 2°C. Instrumental colour, pH and temperature were measured again at 1 and 4 days post slaughter. Cooking loss and WBSF were determined for each ageing period and purge was determined at 4 and 6 days post slaughter. All methods are outlined in chapter two.

Muscle samples (50 ± 10 g) for biochemical assessment were collected at rigor and 4 days post slaughter, frozen in liquid nitrogen and stored at -80°C for assessment within 6 weeks.

Non infused muscles were used as the control as this provides a more realistic comparison to industry practice than that which would be obtained by injecting the controls with water.

4.2.3.2 *Biochemical measurements*

The samples collected and frozen at rigor and 4 days post slaughter were assessed for myofibrillar fragmentation index and sarcomere length using methods described in chapter two.

4.2.4 **Statistics**

The results from these experiments were analysed using the ANOVA function of Genstat 5 (Payne *et al.*, 1988).

The rate of pH and temperature decline and rate of ageing for each treatment were determined by fitting the following equation to the data using the fit curve function of Genstat 5 (Payne *et al.*, 1988):

$$F_t = F_\infty + (F_0 - F_\infty)e^{-kt}$$

where F_t , F_0 and F_∞ are the WBSF/pH/temperature values at time t , at time zero (stunning for pH and temperature decline and rigor for WBSF) and at the completion of ageing/pH/temperature decline, respectively and k is the rate constant. Individual curves were fitted to each muscle and the rate constants were analysed to determine the differences between the treatments. The average curve for each treatment was

determined by fitting the curve to the least squares mean for each time measured and these are presented as the figures in this chapter.

The percentage change from rigor to 4 days post slaughter was determined for WBSF and MFI using the following equations:

$$\begin{aligned} \% \text{ change WBSF} &= \frac{\text{WBSF}_{\text{rigor}} - \text{WBSF}_{\text{4days}}}{\text{WBSF}_{\text{rigor}}} \times 100 \\ \% \text{ change MFI} &= \frac{\text{MFI}_{\text{4 days}} - \text{MFI}_{\text{rigor}}}{\text{MFI}_{\text{4days}}} \times 100 \end{aligned}$$

The data in each experiment was analysed by ANOVA after allowing for the randomisation specified and blocking according to pig. Correlation coefficients were determined between sarcomere length, myofibrillar fragmentation index and WBSF. In experiment 4, the variant function of Genstat 5 was used to determine if a linear or quadratic relationship occurred between the treatments. In addition, in experiment 4 drip loss was analysed after a log transformation to make the error variation more homogenous with increasing values.

In experiment 6 the pH values obtained prior to 6 hours post slaughter were analysed using a modified ANOVA analysis with the 6 hour calcium infusion samples being treated as non-infused muscles prior to 6 hours post slaughter. The highest level of significant interaction is presented in the results section and single effects are only presented when no interactions occurred. Due to negligible calcium by time interactions occurring, the data presented in the tables for calcium chloride infusion is an average for both times.

4.3 RESULTS

4.3.1 Experiment 4. Temperature conditioning of pork following accelerated boning

4.3.1.1 Sample Description

The average live weight of the pigs in this experiment was 98.3 ± 7.60 kg, the average carcass weight was 71.5 ± 6.03 kg and the average P_2 fat measurement was 21.5 ± 3.08 mm.

No muscles could be classified as being PSE using the parameters $L^* > 50$ and drip loss $> 5\%$, ultimate pH < 5.5 (Warner *et al.*, 1993) or DFD using the parameters $L^* < 42$, drip loss $< 5\%$, ultimate pH > 6.0 (Warner *et al.*, 1993).

4.3.1.2 pH and temperature decline

The average pH decline for each treatment can be seen in Figure 4.1. There were no differences in pH between the treatments at 0.5, 1, 3, 5, 10 hours post slaughter, rigor or 4 days post slaughter ($P > 0.05$). At 1.5, 2 and 2.5 hours post slaughter, the muscles chilled at 14 and 21°C had a lower pH than those chilled at 0°C ($P < 0.05$). At 4 hours post slaughter, the muscles chilled at 7°C had a higher pH than all other muscles ($P < 0.01$). By 6 hours post slaughter the 21°C samples had a lower pH relative to the muscles conditioned at 0 or 7°C ($P < 0.05$) while at 8 and 9 hours post slaughter the muscles chilled at 21°C had a lower pH than those chilled at 0 or 7°C ($P < 0.05$). The rates of pH decline determined by fitting exponential decay equations were similar for the four temperature treatments ($P > 0.05$).

The rates of temperature decline were similar for the four treatments ($P > 0.05$) as seen in Figure 4.2. There was no difference in muscle temperature between the different treatments at 30 minutes post slaughter ($P > 0.05$) but by 1 hour post slaughter the muscles chilled at 0 and 7°C had a lower muscle temperature than those chilled at 14 and 21°C ($P < 0.001$). From 1.5 hours post slaughter to rigor there was a significant difference in muscle temperature between all treatments with the muscles chilled at 0°C

having the lowest temperature and those chilled at 21°C the highest ($P < 0.001$ for all times).

4.3.1.3 *Dimensional change, sarcomere length and myofibrillar fragmentation index*

A linear response for the dimensional change was observed with a reduction in dimensional shortening with increasing temperature ($P < 0.05$; Table 4.1). The dimensional change from boning to rigor was higher for the 0°C temperature conditioned treatment relative to muscles conditioned at 21°C ($P < 0.05$).

Sarcomere lengths and myofibrillar fragmentation index (MFI) values at rigor and 4 days post slaughter can be seen in Table 4.1. At rigor ($P < 0.01$) and at 4 days post slaughter ($P < 0.01$) the LTL muscles held at 0°C had shorter sarcomere lengths than all other samples. At rigor, no differences were observed in MFI ($P > 0.05$) but by 4 days post slaughter, the accelerated boned muscles held at 0°C had a lower MFI than the other treatments ($P < 0.01$). There was no differences in the percentage change in MFI from rigor to 4 days post slaughter between the different treatments ($P > 0.05$; Table 4.1).

At rigor, there was a significant correlation between WBSF and MFI ($r = 0.38$; $P < 0.05$) with a decrease in MFI occurring with a decrease in WBSF, however only 15% of the variation was accounted for. At 4 days post slaughter there was a significant correlation between WBSF and MFI; WBSF and sarcomere length and between MFI and sarcomere length ($r = -0.51$; $r = -0.35$; $r = 0.47$ respectively; $P < 0.05$ for all). MFI increased as WBSF decreased and sarcomere length increased; and WBSF decreased as sarcomere length increased.

4.3.1.4 *SDS-PAGE*

A representative SDS-PAGE gel for the different treatments can be seen in Figure 4.3. A comparison of protein degradation in myofibril samples between 1 and 4 days post slaughter for each treatment using SDS-PAGE illustrates that ageing occurred over this time period as indicated by changes to the titin band and the nebulin bands over this

time period. Breakdown of both titin from a singlet in the rigor samples to a doublet in the 4 day samples and nebulin can be observed to occur in all treatments with nebulin disappearing by 4 days. Troponin T appears to have been broken down in all samples by 4 days post slaughter with the formation of the 30kDalton band. Similar patterns to that seen in the representative gel were observed for each treatment in the other gels.

4.3.1.5 *Warner-Bratzler peak shear force*

Temperature conditioning had no effect on WBSF measurements at rigor ($P > 0.05$) as seen in Table 4.1. After 4 days of ageing, a quadratic relationship between WBSF and temperature was seen ($P < 0.05$). The LTL muscles held at 0°C had a higher WBSF than those that were temperature conditioned at 7, 14 and 21°C ($P < 0.01$) and the LTL muscles that were conditioned at 14°C had a lower WBSF than the samples conditioned at 7°C ($P < 0.01$). The percentage change in WBSF from rigor to 4 days post slaughter was influenced by chilling temperature (Table 4.1) with the muscles chilled at 14°C having a greater percentage improvement in WBSF ($P = 0.01$) than all other temperatures.

4.3.1.6 *Cathepsin activity*

Temperature conditioning after accelerated boning did not alter total cathepsin B+L or D activity ($P > 0.05$) but a significant decrease in cathepsin B activity was observed in LTL muscle conditioned at 21°C relative to muscles held at 0, 7 or 14°C ($P < 0.05$) as seen in Table 4.2.

4.3.1.7 *Drip loss and surface colour*

Drip loss, analysed after a log transformation, was increased by temperature conditioning at 0°C and 21°C relative to 7°C and 14°C ($P < 0.01$) (Table 4.3).

The effect of treatment on surface colour at rigor and 4 days post slaughter can be seen in Table 4.3. Temperature conditioning had no effect on L^* at rigor ($P > 0.05$) but at 4 days post slaughter a linear response was observed with the L^* value increasing with increased temperature of conditioning ($P < 0.001$). The LTL muscle held at 0°C

had a lower L^* value than all other samples and the muscles temperature conditioned at 7°C had a lower L^* value than the muscles temperature conditioned at 14 or 21°C. The pH and L^* values at rigor indicate that ultimate pH had not been reached and therefore true rigor mortis had not occurred at the time of measurement.

There were no differences in the a^* values at rigor or after 4 days ageing ($P > 0.05$). The b^* values at rigor and 4 days post slaughter were influenced by the conditioning temperature ($P < 0.01$). At rigor the 0°C temperature conditioned muscles had a lower b^* value than the other three temperature treated muscles. At 4 days post slaughter the 0°C treated muscles had a lower b^* value than the 14 and 21°C treated muscles and the 14°C also had a higher b^* value than the 7°C treated muscles.

4.3.1.8 *Cooking loss and purge*

Temperature conditioning after accelerated boning had a significant effect ($P < 0.01$) on cooking loss at rigor with muscles temperature conditioned at 7, 14 and 21°C having a lower cooking loss than those conditioned at 0°C (Table 4.3). At 4 days post slaughter no difference ($P > 0.05$) between treatments was observed in cooking loss as seen in Table 4.3. Purge levels were higher in the samples temperature conditioned at 0°C than the samples temperature conditioned at 7, 14 or 21°C ($P < 0.01$; Table 4.3).

4.3.2 Experiment 5: Rate of ageing of pork following accelerated boning

4.3.2.1 *Sample description*

The average live weight of the pigs used in this experiment were 89.2 ± 10.91 kg with a carcass weight of 64.6 ± 7.85 kg and a P_2 measurement of 22.8 ± 3.46 mm.

Four muscles (3 in treatment AB-0, 1 in treatment RB) could be classified as being PSE using the parameters $L^* > 50$ and drip loss $> 5\%$, ultimate pH < 5.5 (Warner, 1994). No muscles could be classified as DFD using the parameters $L^* < 42$, drip loss $< 5\%$, ultimate pH > 6.0 (Warner *et al.*, 1993).

The dimensional change of the muscle was similar for the 0°C and 14°C treatments (10.39%, se = 1.84; 6.40%, se = 1.42 respectively, $P > 0.05$).

4.3.2.2 *Rate of pH and temperature decline*

The rates of pH and temperature decline for the three treatments can be seen in Figures 4.4 and 4.5 respectively. At 30 minutes, 1, 2, 4, 5, 6, 7, 8 and 9 hours post slaughter there was no difference in muscle pH between the three treatments ($P > 0.05$). However, at 3 hours post slaughter, the AB-0 muscles had a higher muscle pH than the AB-14 and RB muscles ($P < 0.01$).

At 30 minutes post slaughter, no difference in muscle temperature was observed ($P > 0.05$). By 1 hour post slaughter and continuing to rigor, the AB-0 muscles had a lower temperature than the AB-14 muscles which had a lower temperature than the RB muscles ($P < 0.001$ for all times).

4.3.2.3 *Sarcomere length and myofibrillar fragmentation index*

The effect of treatment on sarcomere lengths and myofibrillar fragmentation index is presented in Table 4.4. At both time periods there was no significant effect of processing method on sarcomere lengths ($P > 0.05$). At rigor the processing method did not influence the myofibrillar fragmentation index ($P > 0.05$) but by 4 days post slaughter the AB-0 muscles had a lower myofibrillar fragmentation index relative to the other two processing methods ($P < 0.01$). There was no difference in the percentage change in MFI ($P > 0.05$) from rigor to 4 days post slaughter between the different treatments.

At rigor, there was no correlation between WBSF, myofibrillar fragmentation index and sarcomere length but by 4 days post slaughter, a correlation between WBSF and MFI ($r = -0.58$); and WBSF and sarcomere length ($r = -0.45$) ($P < 0.05$ for both) was observed.

4.3.2.4 *SDS-PAGE*

A representative SDS-PAGE gel for the different treatments can be seen in Figure 4.6. A comparison of protein degradation in myofibril samples between 1 and 4 days post slaughter for each treatment using SDS-PAGE illustrates that ageing occurred over this

time period as indicated by the following changes. Breakdown of titin from a single band at rigor to a double band by 4 days post slaughter occurred for all treatments. Nebulin and troponin T breakdown was observed in the RB and AB-14 muscles by 4 days post slaughter with the disappearance of the nebulin band and the formation of a 30 kDalton band respectively, but some nebulin and troponin T was still present at 4 days post slaughter for the AB-0 muscles. Similar patterns were observed for each treatment in the other gels.

4.3.2.5 *Ageing rate and Warner-Bratzler peak shear force*

The ageing rate for the three different treatments was determined using the average WBSF values for each time period (Figure 4.7). The AB-14 and RB muscles fitted an exponential decay curve with the rate constants of 0.3 and -0.18 respectively. The ageing rate for the AB-0 treatment could not be fitted to an exponential decay curve or to a straight line with a gradient indicating no ageing has occurred and a straight line parallel to the x axis can be fitted through the mean of 8.74 kg.

There was no effect on WBSF of different treatments at rigor, 18 or 24 hours post slaughter ($P > 0.05$) but by 30 hours the AB-0 muscles had a higher WBSF than the AB-14 muscles ($P < 0.01$, Table 4.5). Also at 30 hours post slaughter, the RB muscles had a lower WBSF than the AB-0. At 2, 3, 4, 6, and 8 days post slaughter the AB-0 muscles had a higher WBSF than the AB-14 samples ($P < 0.05$ for all times). No differences in WBSF were evident at 10 days post slaughter ($P > 0.05$). The percentage change in WBSF from rigor to 4 days post slaughter was not influenced by treatment or within treatment ($P > 0.05$; Table 4.5).

4.3.2.6 *Surface colour, pH and drip loss*

The pH, colour and drip loss at rigor and at 4 days post slaughter are given in Table 4.6. AB-0 muscles had a higher pH ($P < 0.01$) at rigor, a lower L^* value ($P < 0.05$) and an increased drip loss ($P < 0.01$) relative to both RB and AB-14. The RB muscles also had a higher drip loss than the AB-14 muscles ($P < 0.01$). At 4 days post slaughter the RB muscles had a higher pH than the AB-0 and AB-14 muscles ($P < 0.05$) while the

AB-14 muscles had a higher L^* value than the RB and AB-0 muscles at this time ($P < 0.05$).

4.3.2.7 *Cooking loss and purge*

The cooking loss and purge of the LTL muscles appears in Table 4.7. No differences in cooking loss were seen ($P > 0.05$) except at 3 days post slaughter when the AB-0 muscles had a significantly higher cooking loss relative to both the RB and AB-14 muscles ($P < 0.05$). The AB-0 and RB muscles had a higher purge level at both 8 and 10 days relative to the AB-14 samples ($P < 0.05$) while no differences in purge were observed at 4 and 6 days post slaughter ($P > 0.05$).

4.3.3 Experiment 6: Effect of calcium chloride infusion on pork tenderness

4.3.3.1 *Sample description*

The average live weight of the pigs was 89.4 ± 6.16 kg with an average carcass weight and P_2 fat measurement of 61.3 ± 4.92 kg and 20.5 ± 2.54 mm respectively.

The change in weight following infusion of calcium chloride until rigor of the LTL was determined for the accelerated boned muscles. Each LTL was infused to 10% of its original weight but by rigor the LTL was $106 \pm 0.9\%$ of the original weight for the accelerated boned samples infused at 6 hours post slaughter and $104 \pm 1.2\%$ for the accelerate boned samples infused at 0.5 hours post slaughter, regardless of whether they were held at 0°C or 14°C .

Ten muscles could be classified as being PSE (AB-14-Ca6 $n = 2$; AB-14-Ca0.5 $n = 2$; RB $n = 3$; AB-0 $n = 2$; AB-0-Ca0.5 $n = 1$) using the parameters $L^* > 50$ and drip loss $> 5\%$, ultimate pH < 5.5 (Warner *et al.*, 1993). No muscles could be classified as DFD using the parameters $L^* < 42$, drip loss $< 5\%$, ultimate pH > 6.0 (Warner, 1994).

4.3.3.2 *pH and temperature decline*

At 25 minutes post slaughter the pH was lower in the AB-0 and AB-14 muscles relative to the RB muscles ($P < 0.001$). As the 25 min pH readings were performed prior to the 30 min calcium chloride infusion no calcium effect could have occurred.

At 1, 4, 5 and 6 hours post slaughter, calcium chloride infusion reduced the pH relative to the muscles that had not been infused ($P < 0.01$). At 6 hours post slaughter the AB-14 and RB muscles also had a lower pH value than the AB-0 muscles ($P < 0.05$). At 2 and 3 hours post slaughter there was an interaction between processing and calcium chloride infusion on pH with the RB muscles infused with calcium chloride having a lower pH than all other treatments ($P < 0.05$). Furthermore at 2 and 3 hours post slaughter the AB-0, AB-14 and RB muscles had a higher pH than the AB-0-Ca and RB-14-Ca muscles ($P < 0.05$).

There was an interaction ($P < 0.001$) between processing, calcium chloride infusion and time of infusion for the rate of pH decline constant (Figure 4.8). All muscles which were not infused with calcium chloride and those infused at 6 hours post slaughter regardless of the method of processing along with the AB-0-Ca0.5 muscles had a slower rate of pH decline than the AB-14-Ca0.5 and RB-Ca0.5 muscles. Furthermore the RB-Ca0.5 muscles had a faster rate of pH decline than the AB-14-Ca0.5 muscles.

At 25 minutes, 1, 3 and 6 hours post slaughter the AB-0 and AB-14 muscles had a lower temperature relative to the RB muscles as would be expected ($P < 0.001$) (Figure 4.9). The AB-0 muscles were also colder than the AB-14 muscles from 1 hour post slaughter ($P < 0.001$).

4.3.3.3 *Dimensional change, sarcomere length and myofibrillar fragmentation index*

The dimensional change, sarcomere length and MFI results can be seen in Table 4.8. A calcium infusion by time of infusion interaction was observed ($P < 0.05$) with the muscles infused at 6 hours post slaughter having a smaller dimensional change compared to the muscles infused at 0.5 hours post slaughter. The dimensional change could not be determined on the samples that were removed from the carcass at rigor

because of the restraints imposed by the skin. At rigor, the AB-0 muscles had shorter sarcomere lengths relative to the RB and AB-14 muscles ($P < 0.05$). Calcium chloride infusion had no effect on sarcomere length at this time ($P > 0.05$).

By 4 days post slaughter, the AB-0 muscle sarcomere lengths tended to be shorter than the sarcomere lengths of the two other treatments ($P = 0.09$). The calcium chloride infused samples were shorter than those that were not infused ($P < 0.01$). The time of the calcium chloride infusion did not influence sarcomere length ($P > 0.05$).

At rigor no differences were seen in MFI between treatments but at 4 days post slaughter an interaction between processing and calcium chloride infusion was seen ($P < 0.03$). The RB, AB-0-Ca and AB-14-Ca muscles had a lower MFI than the RB-Ca and AB-14 muscles while the AB-14 muscles had a higher MFI than the AB-0 muscles.

No correlations were seen between sarcomere length and WBSF at either rigor or 4 days post slaughter ($r=0.016$; $r = 0.036$ respectively; $P > 0.05$ for both). No correlations were seen between myofibrillar fragmentation index and sarcomere length at rigor or 4 days ($r = 0.041$; $r = 0.152$ respectively; $P > 0.05$ for both); or between myofibrillar fragmentation index and WBSF at either rigor or 4 days post slaughter ($r = 0.173$; $r = 0.069$ respectively; $P > 0.05$ for both).

4.3.3.4 *Warner-Bratzler peak shear force and rate of ageing*

The WBSF results are presented in Table 4.9. Calcium chloride infusion resulted in an improvement in tenderness (reduced WBSF) regardless of the method of processing ($P < 0.001$ for all times). This improved tenderness was observed at rigor when the meat was at its toughest and continued throughout the 6 day ageing period.

At 6 days post slaughter the time of infusion influenced the WBSF values ($P < 0.01$, results not presented). The samples infused at 6 hours post slaughter were more tender than those infused at 0.5 hours post slaughter. The samples infused at 0.5 hours were still more tender than those not infused.

Exponential rates of decay equations were fitted to each sample to determine the rate of ageing (Figure 4.10). The infusion of calcium chloride at either 0.5 hours or 6 hours post slaughter and the method of processing did not influence the rate of tenderisation with no significant differences being observed in the rate constants obtained ($P > 0.05$). The percentage change in WBSF from rigor to 4 days post slaughter was not influenced by treatment method ($P > 0.05$; Table 4.9).

4.3.3.5 *Surface colour, pH and drip loss*

The effects of treatment on meat colour, pH and drip loss at rigor, 1 and 4 days post slaughter can be seen in Table 4.10. For one measurement, drip loss, there was an interaction between processing method, calcium chloride infusion and time of infusion ($P < 0.05$) for which the results are not presented in the table. The interaction was comprised of; the AB-14 muscles without calcium chloride infusion had a lower drip loss than all other treatments while the RB-Ca0.5 muscles had a higher drip loss than all other treatments. In addition for the interaction, the RB, AB-0 and RB-Ca6 muscles had a lower drip loss than AB-0-Ca0.5, AB-14-Ca0.5, AB-0-Ca6 and AB-14-Ca6 muscles.

The AB-0 muscles had a lower L^* value than the AB-14 muscles at rigor ($P < 0.05$). Calcium chloride infused muscles had a higher L^* value than those muscles which were not infused with calcium chloride ($P < 0.05$). At rigor there was no differences in the a^* values ($P > 0.05$) and the AB-14 muscles had a higher b^* value than the RB muscles ($P < 0.01$). Calcium chloride did not affect the a^* or b^* values ($P > 0.05$).

At 1 day post slaughter the accelerated boned muscles temperature conditioned at 14°C had a higher L^* value ($P < 0.05$) than the RB and AB-0 treatments but no differences in a^* or b^* values were observed ($P > 0.05$). At 4 days post slaughter an interaction occurred between processing method, calcium chloride infusion and time of infusion for the L^* value ($P < 0.05$) for which the results are not presented in the table. The interaction is comprised of; the AB-0-Ca0.5 and AB-14-Ca0.5 muscles had a lower L^* value than the RB-Ca0.5, RB-Ca6, AB-0-Ca6, AB-14-Ca6, AB-0 and AB-14 muscles. Furthermore, the AB-0 muscles had a higher L^* value than all other muscles except RB-Ca0.5 muscles. No differences in b^* values were observed between the treatments at 4 days post slaughter ($P > 0.05$) while the AB-0 muscles had a lower a^* value than

the AB-14 muscles ($P < 0.01$) and the muscles infused at 6 hours post slaughter had a lower a^* value than the muscles infused at 0.5 hours post slaughter ($P < 0.01$).

At 4 days post slaughter, calcium chloride infused muscles had a higher pH than the muscles that were not infused with calcium chloride ($P < 0.001$). A similar trend occurred at 1 day post slaughter ($P = 0.07$). There was no processing effect on pH at 1 or 4 days post slaughter ($P > 0.05$ for both).

4.3.3.6 *Cooking loss and purge*

Cooking loss and purge results can be seen in Table 4.11. At rigor, the calcium chloride infused samples had a much higher cooking loss than those that were not infused with calcium chloride ($P < 0.001$). Those infused at 6 hours post slaughter also had a higher cooking loss than those infused at 0.5 hours post slaughter. At 1 and 2 days post slaughter an interaction between processing method and calcium chloride infusion was observed for cooking loss ($P < 0.01$) with the AB-14 not infused with calcium chloride having a lower cooking loss than that of other treatments.

At 4 days post slaughter, no significant effects of treatment on cooking loss were observed but at 6 days the calcium chloride infused samples had a lower cooking loss than those which were not infused with calcium chloride ($P < 0.05$). Overall, there was no clear trend over time of the effects of processing and calcium chloride infusion on the levels of cooking loss.

At both 4 and 6 days post slaughter an interaction between processing method, calcium chloride infusion and time of infusion was observed for purge levels ($P < 0.01$) for which the results are not presented in the table. The main results were that at 4 and 6 days post slaughter, the AB-14 muscles had a lower purge level than all other muscles while the RB-Ca0.5 muscles had a higher purge than all other muscles and at 6 days post slaughter AB-14-Ca0.5 had a higher purge than all other muscles. Furthermore, at 4 days post slaughter, the RB and AB-0 had a lower purge than the RB-Ca0.5, AB-14-Ca0.5, AB-0-Ca0.5, RB-Ca6, AB-0-Ca6 and AB-14-Ca6 muscles while the AB-14-Ca0.5 had a higher purge than all muscles except the RB-Ca0.5 muscles. Also at 6

days post slaughter, the AB-0-Ca0.5, AB-0-Ca6 and AB-14-Ca6 had a higher purge than the RB and AB-0 muscles.

4.4 DISCUSSION

Due to the negative impact of accelerated boning on pork tenderness observed in chapter 3, the research presented in this chapter was designed to determine methods that could overcome this problem. Furthermore, other meat quality attributes were assessed to ensure these factors were not negatively altered. The mechanisms investigated were temperature conditioning, post slaughter ageing and calcium chloride infusion. It was hypothesised that the tenderness of pork following accelerated boning could be improved by either chilling the muscle at 14°C, by post slaughter ageing or by the infusion of calcium chloride.

Tenderness and overall pork quality (in particular, colour and drip loss) is influenced by a number of post slaughter factors. The most important of these post slaughter factors are the rate of pH and temperature decline, the level of proteolytic activity and the amount of protein denaturation that occurs during rigor development. Alterations to any of these factors can have a major impact on pork tenderness and overall pork quality regardless of the time at which the muscle is removed from the carcass.

The rate of pH and temperature decline can influence both tenderness and overall meat quality (Pike *et al.*, 1993; Marsh *et al.*, 1987). The rate of pH decline is influenced by the rate of temperature decline. By decreasing the muscle temperature rapidly, the glycolytic process is slowed down thereby reducing the rate of lactate formation. On the other hand, a very slow rate of temperature decline will accelerate the glycolytic process thereby reducing the muscle pH quickly. However, the rate of pH decline can also be influenced by such factors as preslaughter stress stunning method and stimulation.

The major concern with a fast temperature decline along with a slow pH decline is the occurrence of cold toughening, a common condition in muscles following accelerated boning. Cold toughening involves both cold shortening and the reduction in proteolytic activity due to the conditions induced by cold temperatures. Cold shortening results

from the release of calcium ions from the sarcoplasmic reticulum and mitochondria when the muscle temperature falls below 10°C if the pH is still above 6.0 (Pearson and Young, 1989). The cold conditions reduce the ability for the sarcoplasmic reticulum and mitochondria to reabsorb the calcium and thereby extreme muscle contraction occurs. As the muscles are removed pre-rigor during accelerated boning, cold toughening is the major concern for tenderness and quality characteristics of pork as shown in chapter 3.

Cold shortening influences tenderness. Due to the extensive sarcomere length shortening that occurs during cold shortening, tenderness levels are greatly reduced. Locker (1985) found that in beef a peak toughening occurred with a sarcomere length of 1.3 μm . This level of contraction results in the overlap of A filaments from adjoining sarcomeres (Locker, 1985) resulting in little or no ageing occurring. Ageing is reduced with this level of shortening due to the proteolytic enzymes (calpains and cathepsins) having a low accessibility to the myofibrillar proteins (Iversen *et al.*, 1995) thereby reducing the degradation of myofibrillar proteins which would normally contribute to improvements in tenderness.

Cold shortening also influences both drip loss and colour, increasing drip loss and reducing surface lightness. It is thought that the loss of water from meat originates from volume changes of the myofibril lattice during changes in cross links between actin and myosin at rigor, sarcomere shortening and denaturation of myosin (Judge *et al.*, 1989). The shrinkage of the myofibril lattice resulting from the cross linkage between actin and myosin leads to a greater proportion of free water which can be lost from the meat (Marsh *et al.*, 1972). Thus the higher muscle contraction of cold shortened muscle results in a higher drip loss and moisture loss during storage. The darker colour of cold shortened muscle can be attributed to the reduction in sarcomere length thereby reducing the light scattering ability of the meat, resulting in a darker appearance (Judge *et al.*, 1989).

The level of proteolytic activity is another important factor influencing pork tenderness. Tenderness of pork following accelerated boning is greatly influenced by the activity of the proteolytic enzymes, the calpains and the cathepsins. Like the activity of the glycolytic enzymes, the early activity of both the calpains and the cathepsins is

influenced by the rate of pH and temperature decline. Conditions that are conducive to cold shortening are also likely to reduce the activity of the proteolytic enzymes due to the slow pH decline and rapid temperature decline. On the other hand, a slow temperature decline along with a more rapid pH decline increases the activity of these enzymes (Dransfield, 1994; Zeece and Katoh, 1989). Calpain activity is induced by the release of calcium ions while the temperature is still high while the cathepsins are induced due to the earlier release from the lysosomes due to the fall in pH damaging the lysosomal membranes. Thus the conditions that occur following accelerated boning and chilling at 0°C are likely to inhibit proteolytic activity thereby reducing tenderness. By chilling at higher temperatures and storing the muscles for a period in vacuum bags post slaughter, proteolytic activity should be enhanced.

Storage post slaughter is another factor that can significantly influence pork tenderness, particularly if cold shortening is prevented. An improvement in tenderness due to storage under anaerobic conditions has been attributed mainly to proteolysis resulting in myofibril fragmentation and due to the weakening of the Z discs, increasing sarcomere length. The increase in sarcomere length with post slaughter ageing has been attributed to the weakening of the z disks during ageing as a result of the fall in pH and the presence of calcium ions (Takahashi *et al.*, 1987) and due to the presence of paratropomyosin (Takahashi *et al.*, 1985a and b). As a result of the increase in calcium ion concentrations with rigor in the sarcoplasm, the paratropomyosin is translocated from its original position in the A-I junction of the sarcomere where it performs no known role, onto the thin filament. This translocation of the paratropomyosin weakens the rigor linkages between actin and myosin due to competition with myosin for binding sites to actin. As paratropomyosin binds preferentially to actin, it disengages some of the myosin-actin linkages thereby lengthening the sarcomeres (Hattori and Takahashi, 1988). The storage time and conditions following accelerated boning can greatly influence any subsequent improvements in tenderness during storage.

The impact of the rate of pH and temperature decline and the proteolytic activity on tenderness can be pronounced. Therefore, temperature conditioning, calcium chloride infusion and post slaughter ageing were examined to determine how these altered the rate of pH and temperature decline and the proteolytic activity. The effectiveness of temperature conditioning, post slaughter ageing and calcium chloride infusion on

improving tenderness and maintaining meat quality is outlined in the following discussion.

4.4.1 Experiment 4: Temperature conditioning of pork following accelerated boning

4.4.1.1 *Rate of pH and temperature decline*

The main effect of temperature conditioning of pork following accelerated boning seen in experiment 4 was the prevention of cold toughening. It has been reported that improvements in tenderness only occur when cold shortening is prevented (Dransfield and Lockyer, 1985). Slowing the rate of temperature decline post slaughter by chilling at higher temperatures, will prevent cold toughening conditions due to increases in the rate of pH decline, thereby improving tenderness and overall meat quality. However, temperature conditioning in experiment 4 did not alter the rate constants for pH decline as would be expected with the time taken to reach rigor being 4 hours faster for the muscles chilled at 21°C compared to those chilled at 0°C. However, early pH's at 1.5, 2 and 2.5 hours post slaughter for the muscles chilled at 14 and 21°C were lower than for the muscles chilled at 0 and 7°C. This lower pH was induced by the higher muscle temperature that occurred in the muscles chilled at 14 and 21°C from 1 hour post slaughter. As cold shortening is known to occur under chilling conditions of 0°C, the higher muscle temperature of the muscles chilled at 14 and 21°C avoided cold shortening conditions as originally hypothesised. Furthermore, the higher temperatures would have activated both the glycolytic and proteolytic enzymes.

Dransfield and Lockyer (1985) reported similar results in excised pork *longissimus* chilled at various temperatures between -20 and 20°C to those observed in experiment 4. In the study, Dransfield and Lockyer (1985) reported that samples chilled at 3°C had the slowest decrease in pH taking on average 7 hours to reach pH 6.0 while samples chilled at 20°C reached this pH in 4 hours. However when the rate of pH fall was determined as the fall in pH over 1 to 4 hours post slaughter, no differences in the rate of pH decline were seen between the different chilling temperatures (Dransfield and Lockyer, 1985). Dransfield and Lockyer (1985) explained these results as being due to

the high level of variability observed within treatments especially in those chilled at higher temperatures.

4.4.1.2 *Muscle shortening*

The impact of the rate of temperature decline following temperature conditioning on muscle shortening in experiment 4 is clearly evident with cold shortening being prevented with a higher chilling temperature. The muscles held at 0°C had a shorter sarcomere length than all of the other treated muscles at both rigor and 4 days post slaughter indicating cold shortening. The shorter sarcomere lengths in the AB-0 muscles contributed to a reduction in tenderness as assessed by WBSF and myofibrillar fragmentation index at 4 days post slaughter. Although an increase in sarcomere length was seen from rigor to 4 days post slaughter with muscles chilled at 0°C, it was not sufficient to improve tenderness. Others (Feldhusen and Kuhne, 1992; van Laack and Smulders, 1992) have also observed this increase in sarcomere length in cold shortened muscle with time post slaughter.

The association between sarcomere length shortening and a reduction in tenderness has been extensively examined. Locker (1985) found that in beef a peak toughening occurred with a sarcomere length of 1.3 μm which is the same as the sarcomere length for the muscles chilled at 0°C, explaining the severe toughening observed in this muscle. This level of contraction results in the overlap of A filaments from adjoining sarcomeres (Locker, 1985) resulting in little or no ageing occurring as was seen in the muscles chilled at 0°C. Improvements in tenderness with ageing are also not possible with this level of shortening due to the proteolytic enzymes having a low accessibility to the myofibrillar proteins due to the overlapping of the A filaments of adjoining sarcomeres (Iversen *et al.*, 1995) thereby reducing the degradation of myofibrillar proteins contributing to improvements in tenderness.

The cold shortening determined by sarcomere length shortening was also confirmed by similar results for dimensional shortening. Temperature conditioning at 0°C resulted in considerable dimensional shortening with muscle shortening of 15% occurring in the muscles chilled at 0°C. The same level of shortening was also reported for the muscles following accelerated boning and chilling at 0°C in experiment 3. However, Iversen *et*

al. (1994) observed 27% cold induced shortening in pre-rigor excised pork *longissimus dorsi* muscle when muscles were excised 1 hour post stunning while only 6% when excised at 6 hours. The muscles conditioned at 21°C in experiment 4 obtained a similar value for dimensional shortening (9%) to Iversen *et al.* (1994) samples boned at 6 hours post slaughter (6%).

4.4.1.3 Warner-Bratzler peak shear force

The cold shortening that was observed following chilling at 0°C influenced tenderness improvements with storage of the pork muscle as assessed by WBSF. Tenderness of pork following accelerated boning and temperature conditioning at either 0, 7, 14 or 21°C was not affected at rigor by the temperature at which it was conditioned. However, by 4 days post slaughter the muscles held at 14 and 21°C had a lower WBSF than the muscles that had been held at 0°C. The lack of difference between treatments at rigor was also seen by Marriot *et al.* (1980) who observed no differences in tenderness when pork following accelerated boning was temperature conditioned at 20-25°C. However, the lack of reduction in tenderness of the muscle held at 0°C by 4 days post slaughter relative to the muscles held at 14 and 21°C is consistent with that seen by Marsh *et al.* (1972). Marsh *et al.* (1972) reported that tenderness for pork decreased by only 20-40% when muscles were excised pre-rigor and held at 0°C compared to muscles chilled on the carcass. Thus the shortening observed in the muscles chilled at 0°C was sufficient to prevent improvements in tenderness with ageing which was observed in the muscles chilled at 14 and 21°C as originally hypothesised.

Similar trends for WBSF for muscles following accelerated boning and chilling at 0°C were observed in experiments 3 and 4. The initial WBSF values for the muscles following accelerated boning and chilled at 0°C in experiment 4 were lower than the initial WBSF values of the muscles seen in experiment 3. However, similar WBSF values at 4 days post slaughter were seen for the accelerated boned muscles held at 0°C for both experiments 3 and 4. This difference in the initial values may be explained by the difference in the time of assessment. The muscles in experiment 3 were assessed at 1 day post slaughter while the muscles in experiment 4 were tested at rigor. Thus, further toughening may have occurred between rigor and 1 day post

slaughter in the muscles used in experiment 4 as seen by Wheeler and Koohmaraie (1994) in lamb. The increase in WBSF values from rigor to 1 day post slaughter was also seen to occur in experiments 2 and 5 for the muscles following rigor boning and muscles following accelerated boning and temperature conditioned at 14°C in experiment 5. The increased WBSF values from rigor to 1 day post slaughter may be explained by the fact that the muscle may not have been completely in rigor at the time at which the rigor samples were taken. Therefore further shortening of the muscles occurred between rigor and 1 day post slaughter increasing the WBSF of the muscle.

Differences in the level of ageing that occurred between the treatments can be explained by various combinations of cold shortening, a reduction in proteolytic activity and by protein denaturation as originally hypothesised. No ageing occurred in the AB-0 muscles as indicated by the WBSF values due to both cold shortening thereby inhibition of proteolytic activity and the slower onset of proteolytic activity due to the colder muscle temperature. Little ageing occurred in the AB-7 muscles due to the inhibition of the proteolytic enzymes as suggested by the lower MFI values relative to the AB-14 muscles. The reduction in ageing of the AB-21 muscles can be attributed to protein denaturation as determined by the higher drip loss and lighter surface lightness. Protein denaturation has been reported to be the cause of the lack of improvements in tenderness in PSE pork (Warner *et al.*, 1997) due to denaturation of the myofibrillar proteins and the enzymes themselves. Thus chilling at 14°C provided the ideal conditions to prevent cold shortening, protein denaturation and to promote proteolytic activity. These results are in contrast to those obtained by Parrish *et al.* (1969) with beef. Parrish *et al.* (1969) did not see any improvements in beef muscle tenderness when chilled at either 7 or 15°C for either 24 or 48 hours. However this work by Parrish *et al.* (1969) was compared to muscles chilled at 2°C where cold shortening may not have occurred. The muscles in the study by Parrish *et al.* (1969) were also held on the carcass, which suggests that accelerated boned muscles may behave differently to muscles held on the carcass. It is probable that accelerated boning caused a faster temperature decline and also more shortening due to lack of restraint.

However, similar results for tenderness to those observed in this study following temperature conditioning have been observed in pork by Iversen *et al.* (1994) with delayed boning. Delayed boning results in the muscles being chilled on the carcass for

several hours before boning prior to the onset of rigor. By delaying boning, the rate of temperature decline may be reduced, resulting in similar effects as those seen with temperature conditioning. Iversen *et al.* (1994) found that by delaying boning from 1 hour to 6 hours post slaughter WBSF values were decreased. Furthermore, cold induced shortening as determined by measuring the change in distance between two pins placed in the muscle at boning and measured at 24 hours post slaughter, was reduced from 27.4 to 6.1% by delaying boning to 6 hours post slaughter. The effect of ageing increased as a result of delaying boning with improvements in tenderness from 1 day to 7 days post slaughter for the 6 hour boned samples being 24.5% compared to only 7.3% for those boned at 1 hour post slaughter (Iversen *et al.*, 1994). A similar effect was observed in experiment 4 but with the slower temperature decline being induced by temperature conditioning with 30% improvements in tenderness occurring with chilling at 14°C compared to 0% with chilling at 0°C. Thus the slower rate of temperature decline induced either by temperature conditioning or by delayed boning is reducing the level of muscle shortening and thereby improving tenderness.

4.4.1.4 *Proteolysis*

The impact of chilling conditions on proteolytic activity in this experiment is confirmed by the MFI results at 4 days post slaughter. It is clear from the reduced MFI values for the 0 and 7°C chilled muscles that proteolytic activity is reduced in both the 0 and 7°C chilled muscles as originally hypothesised. From the correlations observed between MFI, sarcomere length and WBSF at 4 days post slaughter, it is clear that both cold shortening and a reduction in proteolytic activity can explain the reduction in tenderness seen in the 0 and 7°C chilled muscles as assessed by WBSF.

In contrast to the MFI data, the reduction in proteolytic activity due to chilling at 0 and 7°C was not reflected in the cathepsin activity or the protein degradation patterns found in SDS-PAGE. However, the impact on cathepsin activity may be masked by the fact that total cathepsin activity was assessed and the amount of cathepsin released from the lysosomes is unknown. Furthermore, the time of assessment may have also masked the results with early differences in cathepsin activity being unknown prior to the onset of rigor. Pre-rigor differences in cathepsin activity have previously been reported by Moeller *et al.* (1976). The changes to titin, nebulin and troponin T from

rigor to 4 days post slaughter indicate that proteolysis occurred in all treatments and was not reduced by chilling at either 0 or 7°C. However, due to the fast ageing time of pork, differences in proteolytic activity may have been missed by assessment at 4 days post slaughter as complete ageing may have occurred by this time. Therefore the reduction in tenderness observed in the AB-0 muscles can be attributed to cold toughening and the reduction in tenderness of the AB-7 muscles can be attributed to a reduction in proteolytic activity.

4.4.1.5 Meat quality

Cold shortening in the muscles chilled at 0°C also influenced the muscle colour and water holding capacity. Temperature conditioning altered the muscle colour only at 4 days post slaughter with the muscles chilled at 7, 14 and 21°C having a higher L* value (lighter colour) than those chilled at 0°C. Furthermore, at 4 days post slaughter the muscles chilled at 7°C had a lower L* value than those held at 14 and 21°C. This lower L* value of the muscles held at 0°C is consistent to that seen in experiment 3 where the surface lightness of the muscles following accelerated boning and chilling at 0°C was darker than the rigor boned muscles. Lighter colour of temperature conditioned carcasses subsequent to accelerated boning has also been observed by Miller *et al.* (1984) and Moller and Vestergaard (1988). This colour difference has been attributed to a higher ultimate pH, faster cooling rate, reduced protein denaturation and shorter sarcomere lengths in muscles that are chilled at 0°C (Shaw and Powell, 1995). The reduction in sarcomere length in the muscles chilled at 0°C reduces the light scattering thereby giving the meat a darker appearance (Judge *et al.*, 1989).

Similar to the impact of chilling temperature on colour, the impact of chilling temperature on water holding capacity can be explained by sarcomere length shortening for muscles chilled at 0°C and protein denaturation for those chilled at 21°C. Drip loss was minimised by temperature conditioning at 7 and 14°C but was increased by conditioning at 21°C. The higher drip loss for the muscles chilled at 21°C may be due to an increase in protein denaturation that would also help to explain the increase in surface lightness. The higher drip loss for the muscles chilled at 0°C maybe due to sarcomere length shortening increasing the density of the myofibrillar matrix and reducing the capacity to hold water.

With regard to minimising drip loss, conflicting conditioning temperatures have been reported for pork (Honikel and Reagan, 1987; Miller *et al.*, 1984; Reagan *et al.*, 1984). The results in experiment 4 conflict with those of Honikel and Reagan (1987) who studied the optimal chilling conditions to conserve the water holding capacity of pork. They found a chilling system which would lead to a meat temperature of either 25°C within three hours post mortem or of 20°C at four hours post mortem would minimise protein denaturation as well as prevent cold shortening. Miller *et al.* (1984) investigating the optimal conditioning time of carcasses following accelerated boning, observed that drip losses were lowest after a three hour conditioning period at 17°C. However, the results of experiment 4 are in agreement with those of Reagan *et al.* (1984) who found that in hot boned primals conditioning of carcasses for three hours at 17°C or for five hours at 11°C was necessary to minimise drip loss.

Similar patterns for drip loss in experiment 4 have also been reported with other species after accelerated boning and temperature conditioning. Studies in turkey thigh showed that the least drip occurred at 12°C (Lesink *et al.*, 1996) and an increased sarcomere length shortening at 0°C that is consistent with the findings here with pork. Furthermore, a similar pattern of drip loss for beef semimembranous muscle was observed after accelerated boning and temperature conditioning (Follett *et al.*, 1974). In this work, Follett and coworkers observed an increase in drip loss with chilling at -5°C and a reduction with chilling at 5, 10 and 15°C with 10°C having the lowest drip loss.

Overall it is clearly evident that cold toughening can be prevented by temperature conditioning at either 14 or 21°C. By preventing cold shortening and promoting proteolysis, improvements in tenderness occur following ageing. However due to the impact of chilling at 21°C on drip loss, protein denaturation and prevention of ageing, 14°C is the preferred temperature to chill pork following accelerated boning to ensure tender meat without detrimentally affecting other meat quality attributes.

4.4.2 Experiment 5: Rate of ageing of pork following accelerated boning

Improvements in meat tenderness have been reported with post slaughter ageing. The tenderisation of meat occurs in two stages - an initial rapid phase followed by a slow

phase (Takahashi, 1996) allowing an exponential decay equation to be fitted to the ageing data. The rapid improvement in tenderness is mainly due to the structural weakening of myofibrils which begins at rigor mortis where tenderness is at its lowest. The slow improvement is due to the structural weakening of the endomysium and perimysium (Takahashi, 1996). However, when severe cold shortening occurs, ageing is reported to be prevented (Iversen *et al.*, 1995; Davey *et al.*, 1967). In a study by Davey *et al.* (1967) the extent of ageing decreased with a reduction in sarcomere length with no ageing occurring in muscles shortened by 40%. The lack of ageing with severe shortening has been attributed to the fact that the proteolytic enzymes are prevented from accessing the myofibrillar proteins (Iversen *et al.*, 1995). Thus, experiment 5 was designed to determine the impact of cold shortening on the ageing rate of pork and to determine the optimal ageing conditions.

4.4.2.1 *Rate of pH and temperature decline*

As in experiment 4, the rate of pH decline was not influenced by the processing method while muscle temperature was lower for the AB-0 muscles by 1 hour post slaughter. However, this lower muscle temperature for the AB-0 muscles resulted in conditions conducive to cold shortening.

Although the rates of pH decline were not altered by the processing method, all muscles were slow to reach rigor and the absolute pH values were lower for higher conditioning temperature at most time points. The muscles in this experiment took on average 11 hours to reach rigor compared to 7 hours in experiment 2. This slow rate of pH decline could help to explain some of the results obtained in this experiment. Furthermore, the majority of AB-0 muscles did not appear to have completely entered rigor mortis at time of assessment with the rigor pH being abnormally high. During the experiment, we assumed that the carcasses must have been DFD, to still be so high in pH at 11 hours post slaughter thus we assumed they were in rigor and removed the muscles accordingly. In retrospect, we should have waited longer to assess the muscles. The pH decline was abnormally slow but others have reported this under conditions of rapid chilling in beef carcasses (Robyn Warner, *pers. comm*) due to the rapid decline in temperature severely slowing the glycolytic rate. Therefore, further

shortening of sarcomeres and toughening of the muscle may have occurred after the initial samples were collected.

4.4.2.2 *Muscle shortening and proteolytic degradation*

Although cold shortening conditions occurred in the AB-0 samples, cold shortening did not seem to occur in contrast to the original hypothesis. This assessment is based upon the absence of difference in both sarcomere length and dimensional change between the different processing treatments. However, this lack of difference may have been due to the fact that the AB-0 muscles may not have been in complete rigor at initial assessment thereby the true sarcomere length and dimensional change measurements were not obtained.

At rigor, there were no differences in sarcomere length, myofibrillar fragmentation index or WBSF between treatments. In addition there was no correlation between these measurements. These results may be due to the AB-0 muscle having not completely entered rigor mortis and thereby further changes in sarcomere length, MFI and WBSF may have occurred and the method of handling (that is cooking for WBSF and freezing for sarcomere length and MFI) may have influenced the results obtained by altering the proteolytic activity of the muscles and the level of muscle shortening.

Increases in sarcomere length with ageing did not result in improvements in WBSF. Sarcomere lengths for the three treatments in experiment 5 increased with 4 days ageing indicating a possible reversibility of the actomyosin complex (Takahashi *et al.*, 1987, 1985a, 1985b). Feldhusen and Kuhne (1992) and Laack and Smulders (1991) have previously reported this increase in sarcomere length with ageing. However, this increased sarcomere length did not result in an improvement in WBSF in the accelerated boned muscle held at 0°C and the rigor boned muscles as would be expected. Both the rigor boned and accelerated boned muscles held at 0°C had a higher WBSF at 4 days post slaughter compared to that seen at rigor. This is in contrast to the accelerated boned muscle held at 14°C where an increase in sarcomere length corresponded to an improvement in WBSF at 4 days post slaughter.

At 4 days post slaughter, again no differences were observed for sarcomere length between the three treatments, however the MFI was reduced in the AB-0 muscles relative to the AB-14 and RB muscles indicating an increase in toughness in the AB-0 muscles. A reduction in proteolytic activity in the AB-0 muscles relative to the AB-14 and RB muscles is also confirmed by the SDS-PAGE patterns with a reduction in protein degradation being observed in the AB-0 muscles from rigor to 4 days post slaughter as indicated by the presence of the nebulin band at 4 days post slaughter. Furthermore, at 4 days post slaughter, the AB-14 muscles had a lower WBSF than the AB-0 and RB muscles, again indicating improvements in tenderness for the AB-14 muscles. The improvements in tenderness in the AB-14 muscles could not be explained by the level of shortening as indicated by sarcomere length and may be due to differences in the level of proteolytic degradation as originally hypothesised with the proteolytic enzymes being activated earlier due to higher chilling temperatures of the AB-14 muscles.

4.4.2.3 *Rate of ageing and Warner-Bratzler peak shear force*

Very different ageing curves were observed for the three treatments as seen in Figure 4.7. The AB-0 treated muscles showed no improvements in tenderness after storage for 10 days while improvements occurred in the RB and AB-14 muscles. This lack of ageing in the AB-0 muscle is consistent with that seen in experiment 4 where no ageing occurred from rigor to 4 days post slaughter in muscles held at 0°C following accelerated boning. No ageing in beef following accelerated boning has also been reported where neck muscle with shortening beyond 20% progressively decreased in the extent of ageing while at 40% shortening no ageing occurred (Davey *et al.*, 1967). Locker (1985) also reported maximum toughening when shortening of 20 to 40% occurred.

The lack of ageing of the AB-0 muscles may also have been due to the muscles not being in rigor at the time of initial assessment. At 30 hours post slaughter, a sharp rise in WBSF occurred for the AB-0 muscles and an exponential decline can be seen beyond this point, fitting the equation: $WBSF = 8.63 + 5.44 e^{-0.74t}$ ($R^2=57\%$, $RSD = 0.61$). This suggests that the muscle may not have been in rigor until 30 hours post slaughter due to the extremely fast rate of temperature decline slowing the rate of

glycolysis. This is also suggested by the higher pH measured at the 12th rib at rigor (pH = 6.24). This very slow rate of pH decline has also been reported at very low temperatures in lamb (Koochmaraie *et al.*, 1996). Furthermore, Dransfield and Lockyer (1985) reported a slow decrease in pH in excised pork muscle when cooled at 3°C, taking 7 hours to reach pH 6.0 relative to 4 hours at 20°C.

Due to the absence of cold shortening, the lack of ageing in the AB-0 muscles was most likely due to changes in early tenderising enzyme activity. Alternatively, the lack of ageing in the AB-0 muscles may be due to the inactivity of the tenderising enzymes early post slaughter. Low temperatures experienced in the early stages post slaughter while the pH decline is occurring could prevent calpain and cathepsin enzymatic activity (Dransfield, 1994; Zeece and Katoh, 1989). Furthermore, changes in tenderness during ageing can be inhibited in muscles with sarcomere shortening which is postulated to prevent enzyme degradation of the myofibrils, due to reduced accessibility to the myofibrils by the enzymes (Iversen *et al.*, 1995) but this would have been expected in all three treatments.

In contrast to the AB-0 muscles, ageing was observed for the AB-14 muscles. The AB-14 muscles had a similar rate constant for the rate of improvement in tenderness with post slaughter storage to that seen for the RB muscles in experiment 2 where 50% of tenderisation also occurred within 2 days post slaughter as originally hypothesised. The similarity in ageing rates can be explained by the chilling conditions post slaughter although the sarcomere length measurements for the AB-0 and AB-14 muscles were not different. The improvement in tenderness with ageing can be explained by a temperature of 14°C occurring at pH 6.0 enabling maximum tenderisation (Honikel and Reagan, 1987) thereby promoting proteolysis and ageing rates comparable to that seen in conventionally boned pork in experiment 2 and by others (Dransfield *et al.*, 1980-81 and Gould *et al.*, 1965).

The rate of ageing for the RB muscle was relatively slow compared to previous reports findings with 50% of improvements in tenderness occurring within 6 days post slaughter. This is in contrast to previous work in pork where 50% of improvements were observed in 2 days (Dransfield *et al.*, 1980-81) while optimum shear force values were reported by Feldhusen and Kuhne (1992) and Bennett *et al.* (1973) for pork by 2

to 3 days post slaughter. These differences in the rate of ageing could possibly be explained by numerous factors such as the size of pigs, breed, stunning method or chilling conditions, all of which can influence tenderness and tenderisation as previously reported in other studies (Petersen *et al.*, 1997; Candek-Potokar *et al.*, 1996; Wood, 1993).

Very different ageing curves were observed for the rigor boned muscles in this experiment compared to experiment 2. The rate constant for the rigor boned LTL muscle in experiment 5 was -0.18 with 50% of tenderisation occurring within 6 days post slaughter. These results are very different to those obtained earlier in experiment 2 where the rate constant was 0.38 and 50% of tenderisation occurred within 2 days post slaughter. Furthermore, the equation determined for the rigor boned muscles in experiment 5 suggests that the initial phase of tenderisation is the slow phase and the second phase is a faster phase. These differences were obtained even after the employment of standardised genetics, transportation, handling, lairage, stunning, slaughtering and chilling conditions.

Differences in pH decline rate between experiments 2 and 5 may explain the differences seen in the rate of ageing of RB pork between these two experiments. Along with the variations in the rate of ageing between the two experiments, variations in the rate of pH decline (0.06 in experiment 5 verses 0.27 in experiment 2) and temperature decline (0.35 in experiment 5 verses 0.41 in experiment 2) were also observed. Thus the RB samples in experiment 2 required 7 hours to reach rigor on average compared with 11 hours in experiment 5. As the conditions prior to and during rigor development are believed to be the most important factors controlling tenderisation and subsequent ageing this could explain the differences observed between the two experiments. The differences in tenderisation due to the conditions during rigor development is believed to be due to variations in rigor development affecting muscle structure, the release of calcium ions from the sarcoplasmic reticulum and the activity of proteolytic enzymes (Dransfield, 1994).

The rates of pH and temperature decline may have been influenced by weather conditions as previously reported in beef (Warner *et al.*, 1986; Ashmore *et al.*, 1973) and lamb (Furnival *et al.*, 1977). Experiment 5 was conducted mid winter when over

night temperature was low compared to experiment 2 in early autumn when overnight temperatures were much higher. Cold stress experienced in overnight lairage with temperatures between 1-5°C relative to the 25°C temperatures during housing at the piggery may have reduced glycogen stores thus slowing the rate of pH decline post slaughter but not to the extent to induce DFD conditions. Warner *et al.* (1986), Ashmore *et al.* (1973) and Furnival *et al.* (1977) have all reported that changes in environmental temperature pre-slaughter may influence ultimate pH in cattle due to a chronic stress response. It is hypothesised that the stress induced by the colder conditions caused shivering which accelerated glycogen metabolism thereby reducing the glycogen stores pre-slaughter resulting in a slower pH decline post slaughter.

In addition, the pigs used in experiment 5 had a smaller live weight and carcass weight than those used in experiment 2. This smaller size probably resulted in the faster temperature decline, reducing the rate of pH decline. As the same chiller was used in both experiments, muscle temperature differences are postulated to be due to differences in carcass size, shape and fatness as previously reported by Marsh (1981) and due to fewer sides being in the chiller in experiment 5 compared to experiment 2 thereby increasing the efficiency of chilling and increasing the rate of temperature decline.

4.4.2.4 Meat Quality

Although cold shortening was not observed in experiment 5, the impact of processing method on colour and water holding capacity was similar to that previously observed in experiments 3 and 4. The lower L* value at rigor in the AB-0 muscles is predominantly due to a difference in the pH at this time with the AB-0 muscle having a significantly higher pH than the RB and AB-14 muscle. The difference in initial meat colour, which disappeared with ageing, was also seen in experiments 3 and 6 and in beef by Shaw and Powell (1995). The changes in colour with time post slaughter may be explained by the drop in pH by 4 days post slaughter and complete onset of rigor relative to assessment at 'rigor'. The initial colour may have been influenced by the muscle not having completely reached rigor mortis at time of assessment which is suggested by the higher muscle pH at rigor.

The AB-0 muscles were seen to have a much higher drip loss when measured at rigor and higher purge levels at 8 and 10 days post slaughter than the other treatments. It is thought that the loss of water from meat originates from volume changes of the myofibrils during changes in cross links between actin and myosin at rigor, sarcomere shortening and denaturation of myosin (Judge *et al.*, 1989). The shrinkage of myofibrils resulting from the cross linkage between actin and myosin leads to a greater proportion of free water which can be lost from the meat (Marsh *et al.*, 1972). Thus the higher muscle contraction of the accelerated boned muscle held at 0°C seen in previous experiments resulted in a higher drip loss and moisture loss during storage. However, the sarcomere length shortening does not explain the increase in drip loss seen in the AB-0 muscles in this experiment as there was no difference in sarcomere length between the three treatments. Honikel *et al.* (1986) also reported increased drip loss in cold shortened pork muscle but only slight affects on tenderness (Reagan and Honikel, 1985). This is in contrast to research by Taylor *et al.* (1980-81) who showed that accelerated boning reduced drip loss in beef after boning at 1-2 hours post slaughter, vacuum packaging and at 3 hours post slaughter placing in a chiller at 10°C for 9 hours then at 1°C for a further 18 hours.

Overall, from this experiment it can be concluded that the processing conditions play an important role in the rate of ageing post slaughter with only the AB-14 muscles ageing as originally hypothesised. The results of this experiment suggest that the rate of pH and temperature decline may influence the subsequent ageing rate. Furthermore, although cold shortening could not be shown to have occurred in the AB-0 muscles, the conditions were still such that tenderness was reduced, drip loss was increased and a darker meat colour resulted. Thus further work is required to address the hypothesis that cold shortening and a reduction in proteolytic activity as a result of lower muscle temperature early post slaughter prevents ageing and to determine the true impact of the rate of pH decline on the rate of ageing.

4.4.3 Experiment 6: Improvements in tenderness following calcium chloride infusion

The final experiment presented in this chapter investigated the impact of calcium chloride infusion on pork tenderness and meat quality. Calcium chloride infusion has

been shown to improve beef and lamb tenderness (Geesink, 1993; Koohmaraie and Shackelford, 1991; Morgan *et al.*, 1991; Wheeler *et al.*, 1991; Koohmaraie *et al.*, 1989). This improvement in tenderness has been attributed to increased activity of the calpain enzymes (Koohmaraie *et al.*, 1989), changes in ionic strength (Wu and Smith, 1987) or by causing severe muscle contraction (Morgan *et al.*, 1991). Due to the effects of calcium chloride on beef and lamb tenderness, it was postulated that calcium chloride could also improve pork tenderness following accelerated and conventional boning. The results in this experiment showed that calcium chloride improved tenderness of pork muscle occurring regardless of the processing method used as originally hypothesised.

4.4.3.1 *Rate of pH and temperature decline*

The results of experiment 6 suggest that one way calcium chloride infusion improves tenderness may be due to altering the rate of pH decline post slaughter. All calcium chloride infused muscles had a higher rate constant for pH decline thus a faster rate of pH decline relative to those that were not infused. Calcium chloride infusion at 0.5 hours post slaughter resulted in a faster pH decline rate relative to those infused at 6 hours post slaughter. This faster rate of pH decline may be attributed to the alteration in ionic strength and calcium concentration. The increased calcium concentration results in an increased activity of the calcium dependent ATPase thereby stimulating the rate of glycolysis due to the increased breakdown of ATP. Thus the faster rate of pH decline may have contributed to the beneficial effect of calcium chloride on pork tenderness. Due to the faster rate of pH decline, proteolytic activity would have been initiated earlier while muscle temperature was higher. Furthermore, the faster rate of pH decline would have prevented cold shortening conditions from occurring.

The influence of calcium chloride infusion on the rate of pH decline has not been widely reported in the literature. Warner (*pers. comm.*) also observed reduced pH at 3 hours post slaughter for pork *longissimus*, *rectus femoris* and *semimembranosus* following calcium chloride infusion. Sodium chloride infusion has been reported to have no influence on ovine *semimembranosus* muscle pH decline following accelerated boning at 3 hours post slaughter relative to muscles not infused following accelerated boning (Stevenson-Barry and Kauffman, 1995).

4.4.3.2 *Sarcomere length and protein degradation*

One mechanism suggested to be the cause of improvements in tenderness following calcium chloride infusion is the massive muscular contraction induced by high calcium concentrations (Morgan *et al.*, 1991). Interestingly, in this experiment calcium chloride infusion did not influence sarcomere length at rigor. Thus it can be concluded that calcium chloride infusion did not induce muscle contraction in contrast to the original hypothesis. Although no difference in dimensional change was observed between muscles which were infused with calcium chloride and those which were not, the time of infusion appeared to alter the dimensional change. Muscles infused at 0.5 hours post slaughter had a greater dimensional change than those infused at 6 hours post slaughter. This suggests that earlier infusion caused greater muscle contraction. However as the impact was not also observed in the sarcomere length measurements, the impact of muscle contraction as a result of calcium chloride infusion did not appear to influence muscle tenderness.

In contrast, at 4 days post slaughter, calcium chloride infused muscles had shorter sarcomere lengths compared with the non-infused muscles, irrespective of the time of infusion. This reduction in sarcomere length due to calcium chloride infusion has also been seen in beef muscle at 24 hours post slaughter along with a much greater level of contraction (Morgan *et al.*, 1991). The low reductions in sarcomere length due to calcium chloride infusion suggests that the improvements in tenderness were not due to massive musculature contraction as proposed by Morgan *et al.* (1991). Thus the improvements in tenderness may be due to either the alteration in ionic strength (Wu and Smith, 1987), the increased activation of the tenderising enzymes (Koochmaraie *et al.*, 1988 and 1989) or the faster rate of pH decline.

The most widely reported mechanism for calcium chloride improving tenderness is by the activation of the tenderising enzymes. However, calcium chloride infusion did not alter the myofibrillar fragmentation index at rigor, suggesting calcium chloride infusion did not influence proteolytic activity at this time disproving the original hypothesis that proteolytic activity would be increased with calcium chloride infusion. However, at 4 days post slaughter, a mixed response to calcium chloride infusion was observed for

the MFI results indicating no clear effect of calcium chloride infusion on proteolytic activity. This contrasts with work in beef where calcium chloride created considerable increases in myofibrillar fragmentation index values relative to control muscles at 3, 9 and 13 days post slaughter (Beekman *et al.*, 1994).

From these results, no clear method of action for calcium chloride infusion to improve tenderness could be determined. The increased rate of pH decline following calcium chloride infusion suggests that this and an alteration to ionic strength may contribute to the improvements in tenderness. Furthermore, the shorter sarcomere length following calcium chloride infusion by 4 days post slaughter suggests that some alterations to muscle contraction may have occurred. Finally, no clear pattern of MFI was observed to occur following calcium chloride infusion thus it can not be said that increased proteolysis is the cause of the tenderness improvements. Therefore, it is suggested that the improvements in tenderness following calcium chloride infusion observed here resulted from a combination of mechanisms.

4.4.3.3 *Rate of ageing and Warner-Bratzler peak shear force*

Tenderness of muscles following calcium chloride infusion was improved regardless of the processing method as originally hypothesised. Calcium chloride infusion following rigor boning has previously been reported in lamb (Koochmaraie and Shackelford, 1991; Koochmaraie *et al.*, 1990; Koochmaraie *et al.*, 1989; Koochmaraie *et al.*, 1988b), and in pork boned at 1 day post slaughter following blast chilling (McFarlane and Unruh, 1996). In the study by McFarlane and Unruh (1996), infusion of calcium chloride occurred at 24 hours post slaughter and tenderness was reported to improve after 3 days storage at 1°C. As initial tenderness was not reported, it is not known if 3 days storage would be required after infusion at 24 hours post slaughter, which would add to production costs. The need for post slaughter storage can be eliminated by infusing at either 0.5 hours or 6 hours post slaughter as was seen in experiment 6. Infusion of calcium chloride at 24 hours to reduce WBSF has also been reported in beef (Boleman *et al.*, 1995; Wheeler *et al.*, 1993). As beef is usually stored for at least 3 days prior to consumption, infusion of calcium chloride at this time is a much more practical option. As pork is generally not stored for very long prior to consumption (2-3 days) earlier infusion of calcium chloride would be more practical.

Unlike the results seen in experiment 6, the time of calcium chloride infusion has previously been reported to influence tenderness. For example, Boleman *et al.* (1995) found that beef *semimembranous* muscles infused at 1 hour post slaughter had the lowest shear force values indicating that infusion early post mortem is most effective in reducing shear force relative to infusion at either 12 or 24 hours post slaughter. Although not as effective, Boleman *et al.* (1995) reported calcium chloride infusion at 12 and 24 hours reduced shear force by 10.5 and 9.4% respectively relative to those not infused (improvements of 28.6% in shear force were seen in samples infused 1 hour post slaughter). Infusion at 12 hours post slaughter in beef muscle by Boleman *et al.* (1995) may be comparable to infusion at 6 hours post slaughter in pork due to the variation in rate of glycolysis between beef and pork. As pork reaches rigor by approximately 5 to 6 hours post slaughter and beef by 10 to 12 hours post slaughter, infusion at 6 hours for pork and 12 hours for beef correspond approximately to the onset of rigor. Thus unlike the results seen here in pork, beef did not improve in tenderness as much with calcium chloride infusion at rigor compared to infusion at slaughter (Boleman *et al.*, 1995). Wheeler *et al.* (1993) also reported a difference between times of infusion in beef in the effectiveness of calcium chloride infusion on reducing WBSF. They reported the *semimembranous* and *triceps brachii* muscles to be more tender when infused at 24 hours post slaughter relative to infusion at 30 minutes post slaughter. However Wheeler *et al.* (1993) reported no differences in tenderness between muscles injected with calcium chloride at 30 minutes or 24 hours post slaughter for the *longissimus dorsi*, a similar result to that seen here in pork when infusion occurred at either 30 minutes or 6 hours post slaughter.

Calcium chloride infusion in this experiment was seen to be as effective in improving pork tenderness as ageing at 2°C for 6 days when calcium chloride infusion was not applied. The WBSF values were similar to the control samples at 6 days post slaughter and calcium chloride infused samples at 1 day post slaughter. This effect has also been observed in beef where infusion at 1 hour post slaughter was a more effective means of improving tenderness than ageing for 10 days (Boleman *et al.*, 1995). The use of ageing for 10 days and calcium chloride infusion resulted in even greater improvements in tenderness than seen in muscles which were not infused with calcium and aged for 10 days (Boleman *et al.*, 1995) as also seen in experiment 6 for pork.

Thus calcium chloride infusion eliminates the need for ageing thereby reducing production costs.

Although accelerated boning and temperature conditioning at 0°C did not seem to decrease tenderness in this experiment, in previous work in pork (experiments 3 and 4, Woltersdorf and Troeger, 1987) considerable tenderness problems have resulted following accelerated boning and chilling at 0°C. When reductions in tenderness are observed with accelerated boning, calcium chloride would be a very successful method to overcome reductions in tenderness. Thus, calcium chloride infusion allows accelerated boning to be successfully employed due to improvements in tenderness. Furthermore, calcium chloride infusion of post rigor boned muscles can also ensure improvements in tenderness and reduced storage time required for optimal tenderness. From these results calcium chloride infusion appears to result more consistently in improving tenderness than temperature conditioning at 14°C. Temperature conditioning does not seem to be necessary when calcium chloride infusion is administered after accelerated boning making chilling regimes easier to control. However, due to the expense involved in calcium chloride infusion, temperature conditioning at 14°C would be a cheaper option to improve tenderness of pork following accelerated boning.

In addition to improvements in tenderness following calcium chloride infusion, the time required to age pork was reduced to 1 day post slaughter. Calcium chloride infusion reduced the time necessary for ageing to only 1 day compared to non infused pork which took 4 days for 80% ageing in experiment 2 and even longer if extreme cold shortening occurred as seen in experiment 5. This reduction in time required for ageing can be explained by the initial WBSF values being lower thus the relative improvement in tenderness required for acceptable tenderness is reduced. Also the rate of ageing could be improved due to the increased activation of the calpain enzymes by calcium chloride as reported by Koohmaraie *et al.* (1988). This reduction in ageing time following calcium chloride infusion has also been reported in lamb (Koohmaraie *et al.*, 1990) and beef (Wheeler *et al.*, 1991) where only 1 day was required for both species to ensure tender meat. Even after 14 days of ageing in beef, calcium chloride infused meat was more tender than the control (Wheeler *et al.*, 1991). Wheeler *et al.* (1991)

reported that accelerated boning and chilling at 2°C did not influence peak shear force in beef which is a similar result to that observed in experiment 6.

However, no differences in the rate of ageing or percentage change in WBSF from rigor to 4 days post slaughter was observed between the treatments in experiment 6. This further suggests that the addition of calcium chloride did not increase the activation of the tenderising enzymes. The absence of a faster ageing rate due to calcium chloride infusion contrasts with the results of Wheeler *et al.* (1991) for beef. This may be due to the rate of ageing of pork being much faster than that reported for beef (Dransfield *et al.*, 1980). The impact of calcium chloride infusion on the rate of ageing may be too small to measure as both infused and non infused muscles had a reduction in WBSF from rigor to 6 days post slaughter of only 2.4 kg.

Conflicting rates of ageing for the RB, AB-0 and AB-14 muscles were observed compared to those observed in experiment 5. The rate of ageing for the RB muscles in experiment 6 was faster than that previously seen, but similar to that seen by Bennett *et al.* (1973). Bennett *et al.* (1973) also found that ageing past 2 days post slaughter did not improve the palatability of pork. Furthermore, the AB-0 muscles in experiment 6 were observed to improve in tenderness with post slaughter storage, in contrast to the lack of ageing seen for the AB-0 muscles in experiment 5. This difference cannot be explained by sarcomere length shortening as the sarcomere lengths seen in the AB-0 muscles in experiment 6 were slightly shorter than those seen in experiment 5 (1.40µm in experiment 6 verses 1.49 µm in experiment 5). Finally, a faster rate of ageing in experiment 6 for the AB-14 muscles occurred relative to experiment 5. Again this difference in the rate of ageing seen between experiments 5 and 6 may be explained by the differences in rate of pH decline post slaughter. The rate of pH decline in experiment 6 was faster than that observed in experiment 5 thus the lower pH while carcass temperature was still high would have promoted the activity of the tenderising enzymes thereby increasing the rate of ageing.

4.4.3.4 Meat quality

Due to differences in sarcomere length as a result of processing method, once again differences in meat colour and water holding capacity were observed between the

different processing methods. At rigor, the AB-0 muscles had a lower L* value than the AB-14 muscles. This difference in colour between the AB-0 and AB-14 muscles has been previously seen in pigs in experiment 4 and in beef (Shaw and Powell, 1995). This difference can be attributed to the differences in pH and temperature decline post slaughter. The higher L* value seen in the AB-14 muscles after ageing for 4 days was also seen in experiment 4. Once again drip loss was reduced in the AB-14 muscles. The chilling temperature preventing both cold shortening and protein denaturation, both of which are associated with increased drip loss, can explain the lower drip loss of AB-14 muscles.

Although improvements in tenderness were observed following calcium chloride infusion, calcium chloride infusion had a negative impact on pork colour and water holding capacity which resulted in a high number of PSE carcasses following calcium chloride infusion. Calcium chloride infused muscles had a higher L* value and hence lighter than the non infused muscles in experiment 6. Wheeler *et al.* (1993) reported a darker colour due to calcium chloride infusion in beef after 3 days in a retail display. The lighter colour in experiment 6 resulting from calcium chloride infusion may be due to alterations in the interaction between actin and myosin due to changes in the density of the muscle fibres as a result of muscle contraction thereby altering the light scattering properties of the muscle. Due to changes in the ionic strength of the muscle induced by calcium chloride infusion, the binding properties of the actin and myosin may be altered thus altering the muscle colour. The increased moisture content of the muscle resulting from infusion of calcium chloride may also alter the colour. The increased water content could be increasing the light reflectance of the muscle in a similar manner to that which is observed in PSE pork (Judge *et al.*, 1989).

High purge levels are detrimental to retail sales. Higher purge levels were seen in the muscles following accelerated boning and holding at 0°C in experiments 4, 5 and 6 and in rigor boned muscles in experiment 5. The increased purge in the muscles following accelerated boning and chilling at 0°C further indicates the reduced water holding capacity of these muscles as a result of the reduction in sarcomere lengths due to the rapid fall in muscle temperature while muscle pH was above 6.0. This increased purge in the accelerated boned muscle held at 0°C is in contrast to that seen in beef after accelerated boning and chilling at 1°C (Shaw and Powell, 1995). The differences in

purge between beef and pork may be due to the different handling methods. The beef was boxed and cooled in chillers rather than in iced water baths (Shaw and Powell, 1995). However an increase in purge levels was reported in beef in samples following accelerated boning and chilling at -5°C relative to samples chilled at 5, 10 and 15°C (Follett *et al.*, 1974).

Furthermore, calcium chloride infusion increased drip loss. The increased drip loss in the calcium infused muscles relative to those that were not infused can be attributed to the extra moisture added to the muscle with infusion and has previously been reported (Boleman *et al.*, 1995). The amount of drip loss may have also been increased due to changes in the ionic strength of the muscle as a result of calcium chloride infusion. The change in ionic strength may alter the affinity of the proteins to bind to free water molecules thereby increasing the level of drip loss. The increased drip loss as a result of calcium chloride infusion has also been reported in beef *semimembranous* muscle after infusion with calcium chloride. Drip loss was increased regardless of whether the infusion occurred at 1, 12 or 14 hours post slaughter (Boleman *et al.*, 1995). The high level of drip loss in the rigor boned muscle infused at 0.5 hours post slaughter may be due to more than 10% volume being infused into the muscle. This could have occurred because the weight of infusion was calculated on the weight of the muscle removed 0.5 hours post slaughter from the other side of the carcass. Thus temperature conditioning is a more effective way to reduce drip loss after accelerated boning than calcium chloride infusion due to the increased drip loss with infusion.

The change in water holding capacity following calcium chloride infusion is further reflected in the cooking loss results. Cooking loss was observed to increase with calcium chloride infusion up to 1 day post slaughter and again at 6 days post slaughter. Cooking loss has previously been shown to be greater in beef *biceps femoris* due to calcium chloride infusion but not in the *semimembranous* muscle (Wheeler *et al.*, 1991). These trends for the *semimembranous* muscle remained consistent with time (Wheeler *et al.*, 1991) unlike the inconsistencies seen for pork LTL muscle in experiment 6. Cooking loss at either 1, 12 or 24 hours post slaughter has also been seen to increase with calcium chloride infusion in beef *semimembranous* muscle (Boleman *et al.*, 1995). These differences in cooking loss due to calcium chloride infusion, like drip loss, may be attributed to the extra moisture retained due to infusion

of calcium chloride solution. In contrast, no differences in cooking loss were reported in lamb carcasses after calcium chloride infusion (Koohmaraie *et al.*, 1990) or in post rigor boned pork loin infused at 24 hours post slaughter (McFarlane and Unruh, 1996).

Purge levels were also increased with calcium chloride infusion. Furthermore the muscles infused at 0.5 hours post slaughter had a higher purge level than those infused at 6 hours. This higher purge level with infusion can be attributed to the higher moisture content of the meat due to infusion of the calcium chloride. However the increased purge as a result of calcium chloride infusion is in contrast to that seen in pork *longissimus* muscle infused at 24 hours post slaughter by McFarlane and Unruh (1996) and for beef *semimembranosus* muscle infused at 1, 12 or 24 hours post slaughter by Boleman *et al.* (1995). In the study by McFarlane and Unruh (1996), they reported that infusion of post rigor boned muscle at 24 hours did not increase either cooking loss or purge after meat was aged for 3 days at 1°C. This lack of difference in the study by McFarlane and Unruh (1996) in purge and cooking loss was reported to be due to the large variation within the treatment groups masking any differences.

4.5 CONCLUSIONS

From these experiments it is clear that the detrimental effects of accelerated boning seen in experiment 3 on pork tenderness can be overcome with either calcium chloride infusion or temperature conditioning at 14°C and ageing for 4 days post slaughter as originally hypothesised. Calcium chloride infusion produced the most tender pork, requiring less than 1 day of ageing to achieve consumer acceptable tenderness levels. Although the initial and ultimate tenderness were both improved by calcium chloride infusion regardless of the method of processing, the rate at which the muscles aged was not improved by infusion and other meat quality attributes were adversely affected.

Muscle tenderness is considered to be acceptable to the consumer only when a WBSF value of less than 5 kg is obtained (Shorthose *et al.*, 1986). Thus, calcium chloride infusion was the only treatment in these studies that produced consumer acceptable tenderness. Regardless of processing method, the calcium chloride infused muscles reached this tenderness level after 4 days storage post slaughter. Thus it can be concluded that calcium chloride infusion is required regardless of the method of

processing to ensure that a consistent consumer acceptable product is produced. A minimum of 4 days ageing should be employed and temperature conditioning on its own is not an effective method to consistently produce consumer acceptable pork. However, care must be employed with calcium chloride infusion to ensure surface colour and drip loss are not adversely affected. Further investigation is required to determine the conditions that can be employed that will not adversely affect drip loss and colour. Although not as effective at improving tenderness, temperature conditioning at 14°C does not negatively impact on the water holding capacity of the muscles.

The improved tenderness following temperature conditioning at 14 and 21°C in experiment 4 can be attributed to the prevention of cold toughening as originally hypothesised. Chilling of muscles at 0°C following accelerated boning results in cold shortening as seen by the reduction in sarcomere length relative to muscles chilled at 14 or 21°C. The cold shortening prevented proteolysis thereby preventing improvements in tenderness with post slaughter storage. Furthermore, the reduced sarcomere length increased drip loss and produced pork of a darker colour. The reduction in tenderness in the muscles chilled at 7°C can be attributed to a reduction in proteolytic activity. Finally the lower percentage change in WBSF from rigor to 4 days post slaughter for the muscles chilled at 21°C may be attributed to protein denaturation as indicated by the pale surface colour and the increased drip loss.

Calcium chloride infusion was shown to be an effective method of overcoming tenderness problems in pork regardless of the processing method. However, the time of infusion did not seem to influence tenderness results. The improved tenderness observed following calcium chloride infusion in experiment 6 may be attributed to a number of factors. The faster rate of glycolysis induced by calcium chloride infusion may have contributed to the improvements in tenderness. Furthermore changes in the ionic strength and level of muscular contraction may also have contributed to improvements in tenderness. However as the rate of ageing or percentage change in WBSF from rigor to 4 days post slaughter was not altered, the increased activation of calpain enzymes as a result of calcium chloride infusion did not seem to play an important role in improving pork tenderness.

Finally, post slaughter storage of pork appears to be effective at improving tenderness when severe chilling conditions are prevented. Under conditions that could result in cold shortening, ageing was prevented due to the shortening of the sarcomeres preventing proteolysis from occurring. As the AB-0 muscles did not appear to have completely entered rigor mortis at initial sampling as indicated by the higher muscle pH, the true impact of this treatment on sarcomere length may have been missed.

Thus, from these experiments it is clear that the need to prevent cold shortening conditions is required to maximise pork tenderness.

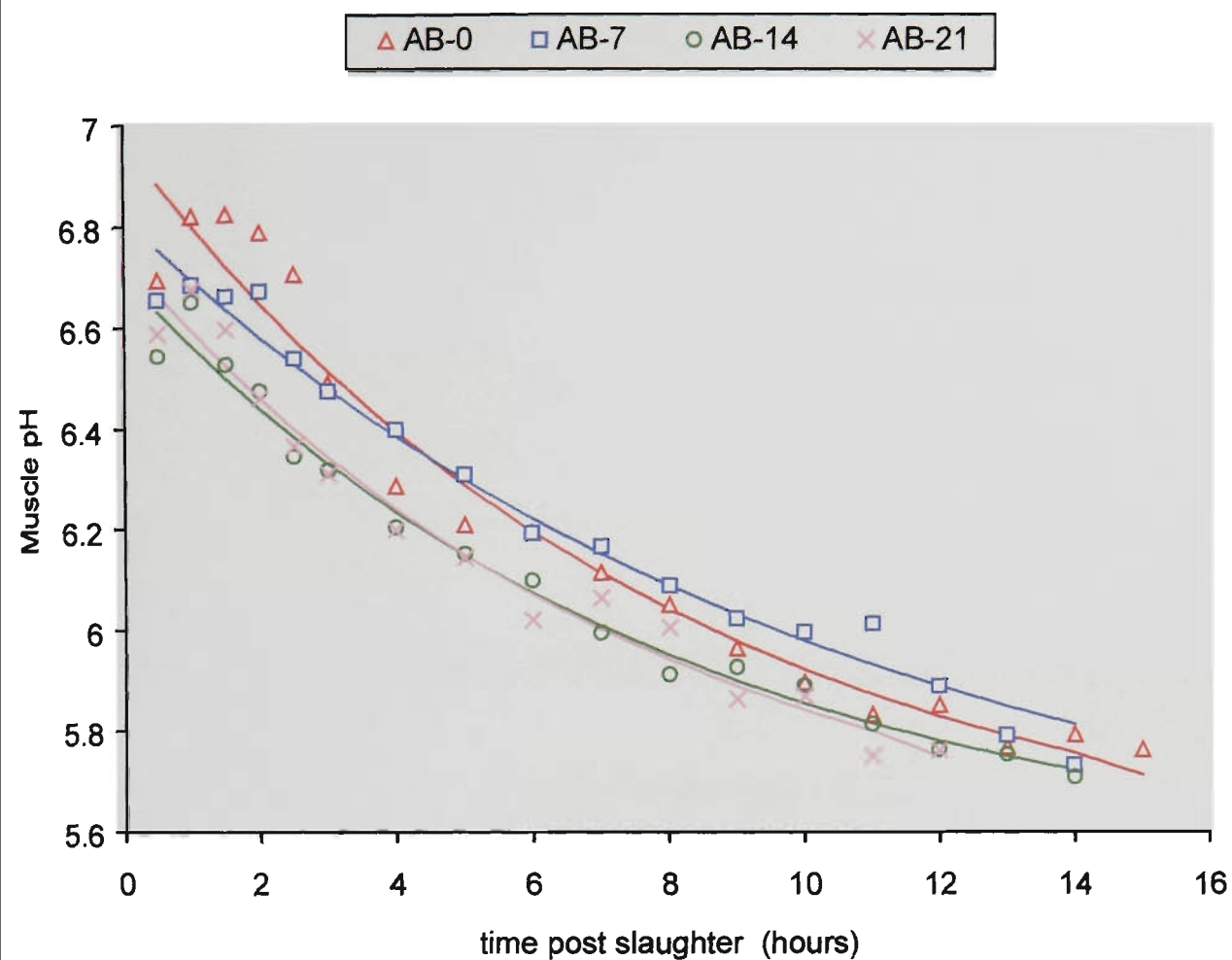


Figure 4.1 Average rate of pH decline in experiment 4 of pork *M. longissimus thoracis et lumborum* after accelerated boning and temperature conditioning in water baths at 0 (AB-0), 7 (AB-7), 14 (AB-14) or 21°C (AB-21) from 0.5 hours post slaughter rigor.

The equations for each line are:

$$pH_{AB-0} = 5.50 + 1.47 e^{-0.13t} (R^2 = 96\%, RSD = 0.08),$$

$$pH_{AB-7} = 5.47 + 1.35 e^{-0.10t} (R^2 = 98\%, RSD = 0.05).$$

$$pH_{AB-14} = 5.53 + 1.18 e^{-0.13t} (R^2 = 99\%, RSD = 0.04) \text{ and}$$

$$pH_{AB-21} = 5.51 + 1.23 e^{-0.13t} (R^2 = 98\%, RSD = 0.05).$$

The average SED = 0.09.

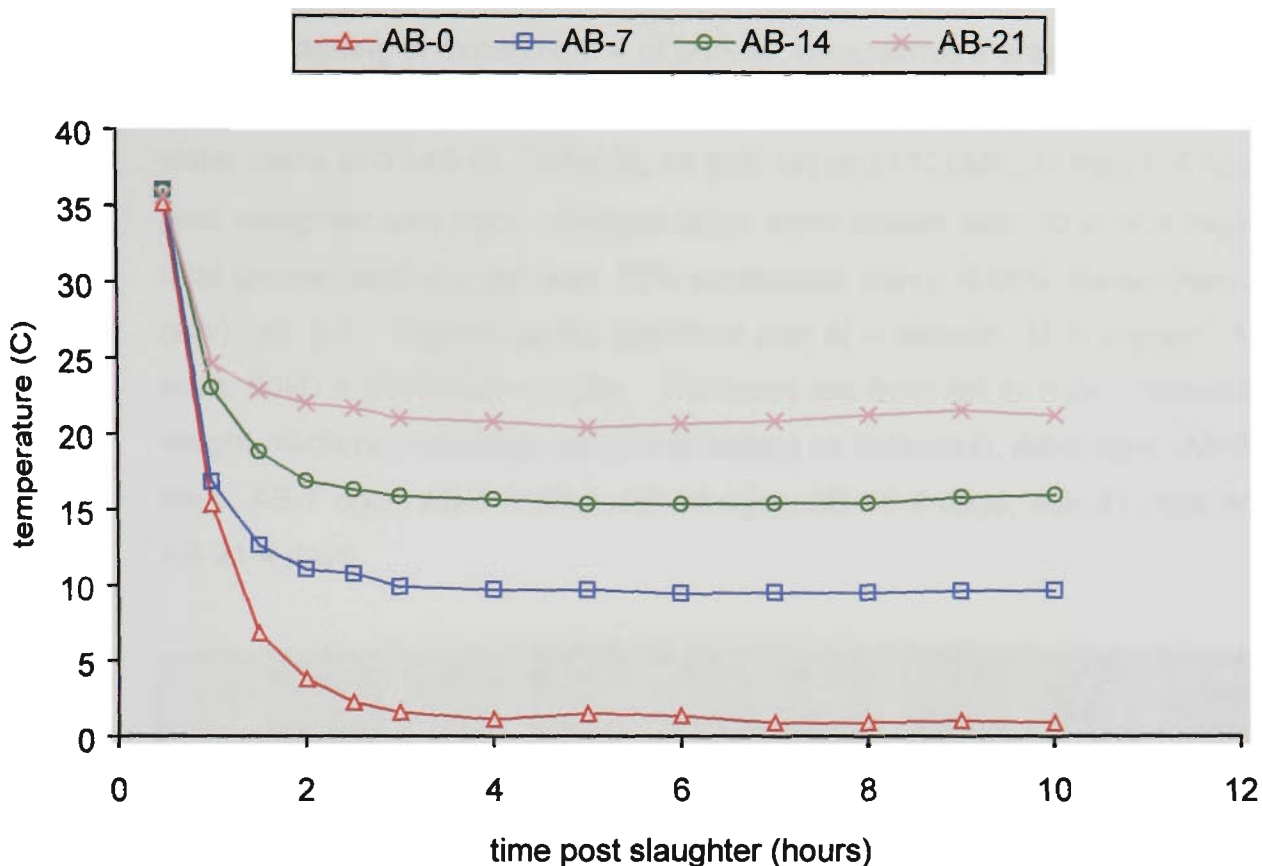


Figure 4.2 Average rate of temperature decline in experiment 4 of pork *M. longissimus thoracis et lumborum* after accelerated boning and temperature conditioning in water baths at 0 (AB-0), 7 (AB-7), 14 (AB-14) or 21°C (AB-21) from 0.5 hours post slaughter until rigor.

The equations for each treatment are:

$$\text{temp}_{\text{AB-0}} = 0.34 + 41.57 e^{-0.96t} \quad (R^2 = 93\%, \text{RSD} = 0.21),$$

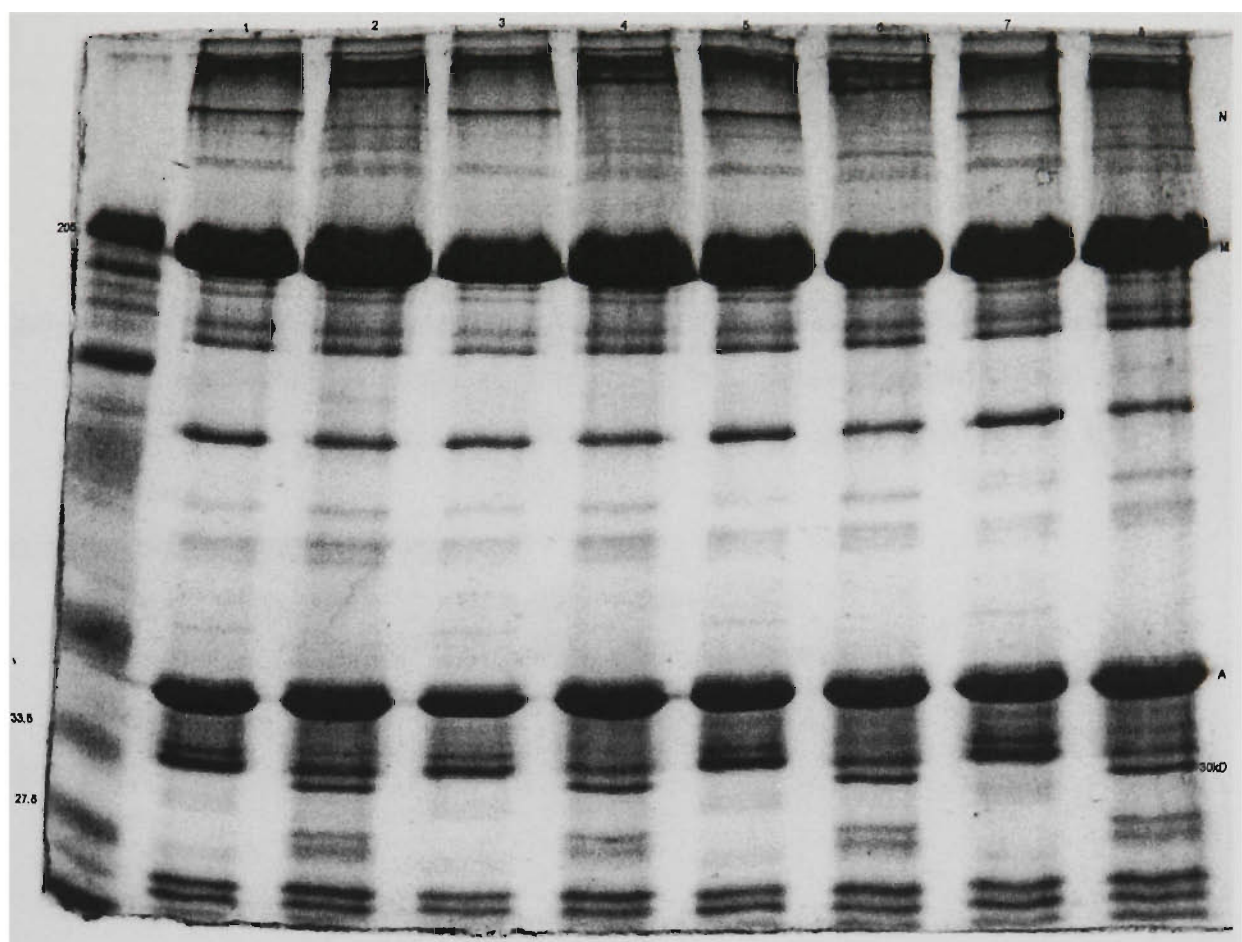
$$\text{temp}_{\text{AB-7}} = 9.18 + 31.79 e^{-1.09t} \quad (R^2 = 91\%, \text{RSD} = 0.43),$$

$$\text{temp}_{\text{AB-14}} = 15.31 + 24.95 e^{-1.09t} \quad (R^2 = 93\%, \text{RSD} = 0.37) \text{ and}$$

$$\text{temp}_{\text{AB-21}} = 20.71 + 18.73 e^{-1.14t} \quad (R^2 = 92\%, \text{RSD} = 0.44).$$

The average SED = 0.54

Figure 4.3 Representative SDS-PAGE showing the difference in protein bands following ageing in experiment 4 of pork *M. longissimus thoracis et lumborum* myofibrils after accelerated boning (AB) and temperature conditioning in water baths at 0 (AB-0), 7 (AB-7), 14 (AB-14) or 21°C (AB-21) from 0.5 hours post slaughter until rigor. Sample lanes were loaded with 10 µl of 4 mg/ml total protein and the gel was 10% acrylamide (w/v), 0.06% bis-acrylamide (w/v), pH 9.3. Protein bands identified are; N = nebulin, M = myosin, A = actin, 30kD = 30 Kdalton protein. The lanes are from left to right – molecular weight markers (molecular weights (kDalton) as indicated), AB-0 rigor, AB-0 4 days, AB-7 rigor, AB-7 4 days, AB-14 rigor, AB-14 4 days, AB- 21 rigor and AB-21 4 days.



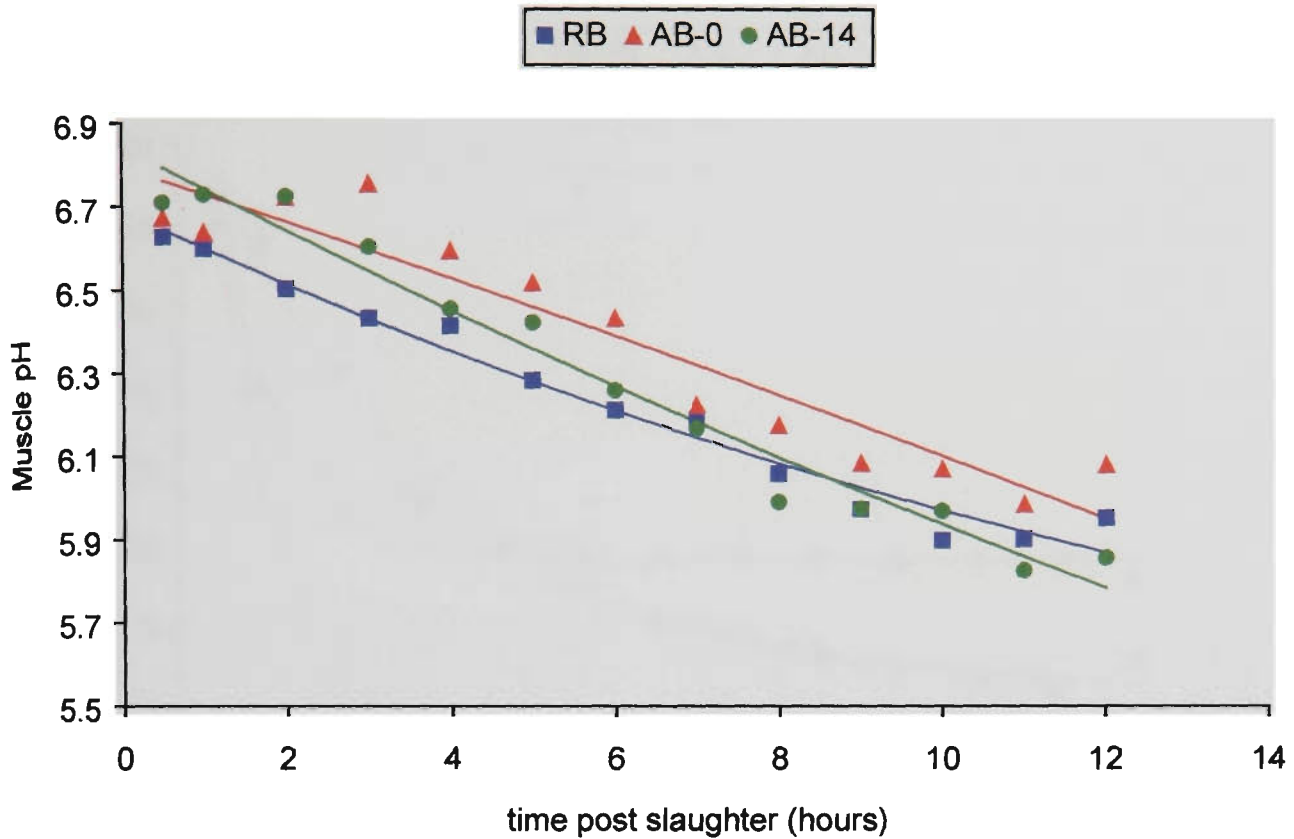


Figure 4.4 Average rate of pH decline of pork in experiment 5 *M. longissimus thoracis et lumborum* after rigor boning (RB), accelerated boning and chilling at 0°C (AB-0) and accelerated boning and temperature conditioning at 14°C (AB-14).

The equations for each treatment are:

$$pH_{RB} = 5.01 + 1.68 e^{-0.06t} (R^2 = 97\%, RSD = 0.05),$$

$$pH_{AB0} = 11.1 - 4.3 e^{+0.02t} (R^2 = 88\%, RSD = 0.10) \text{ and}$$

$$pH_{AB14} = 3.08 + 3.77 e^{-0.04t} (R^2 = 96\%, RSD = 0.06).$$

The average SED = 0.07.

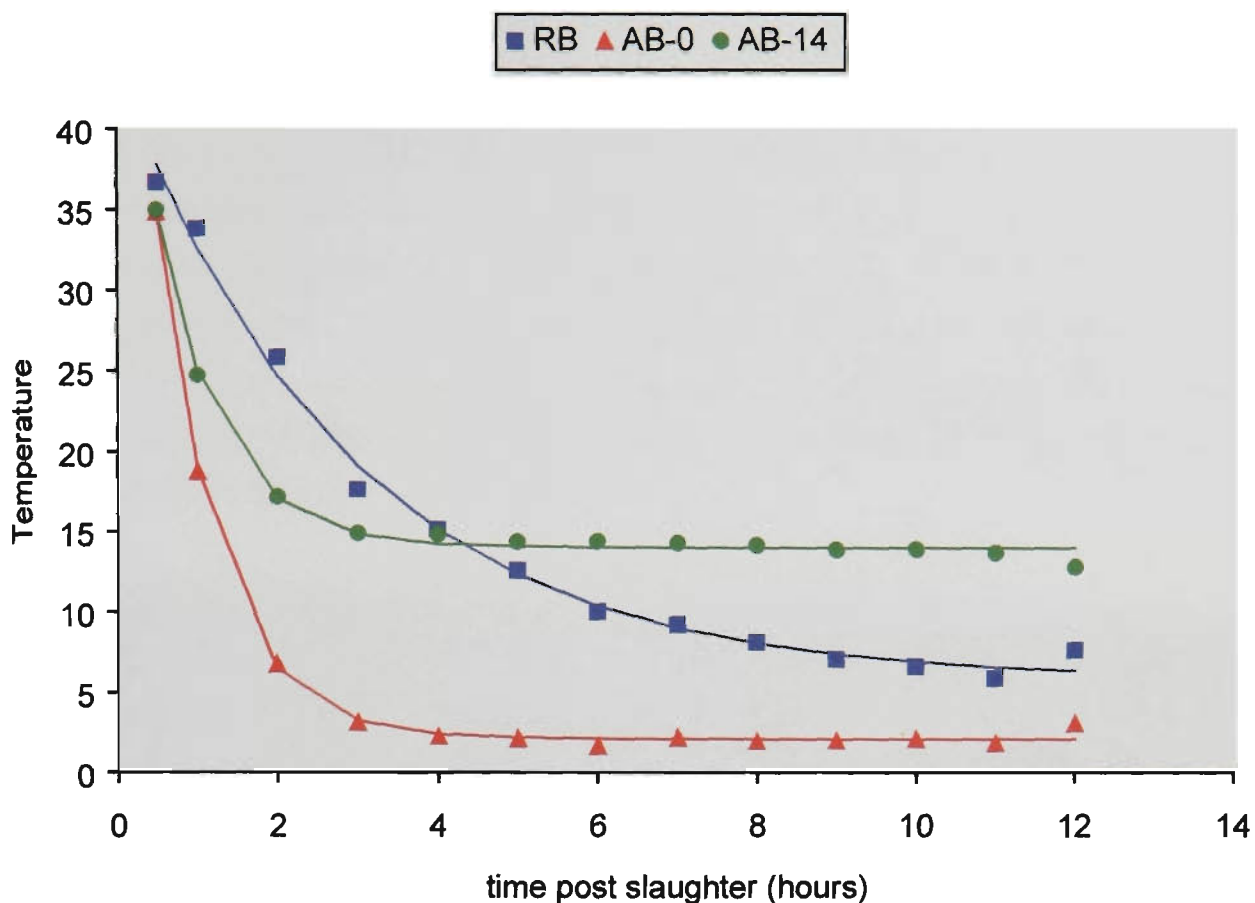


Figure 4.5 Average rate of temperature (°C) decline in experiment 5 of pork *M. longissimus thoracis et lumborum* after rigor boning (RB), accelerated boning and chilling at 0°C (AB-0) and accelerated boning and temperature conditioning at 14°C (AB-14).

The equations for each treatment are:

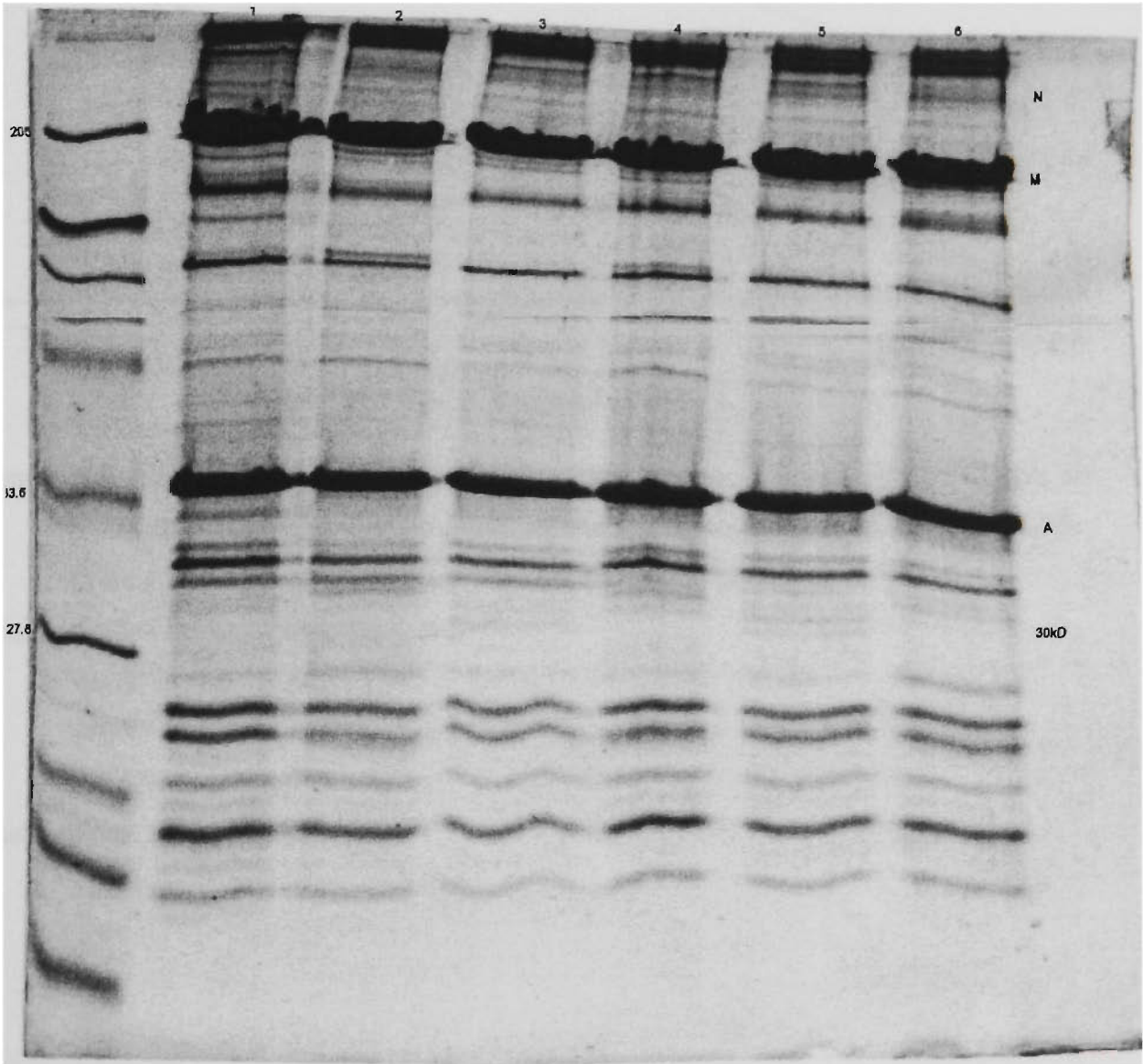
$$\text{temp}_{\text{RB}} = 5.78 + 32.02 e^{-0.35t} \quad (R^2 = 99\%, \text{RSD} = 0.94),$$

$$\text{temp}_{\text{AB0}} = 2.08 + 32.76 e^{-1.34t} \quad (R^2 = 99.9\%, \text{RSD} = 0.37) \text{ and}$$

$$\text{temp}_{\text{AB14}} = 14.00 + 20.89 e^{-1.29t} \quad (R^2 = 99.5\%, \text{RSD} = 0.46).$$

The average SED = 1.03.

Figure 4.6 Representative SDS-PAGE showing the difference in protein bands following ageing in experiment 5 of pork *M. longissimus thoracis et lumborum* myofibrils after rigor boning (RB), accelerated boning and chilling at 0°C (AB-0) and accelerated boning and temperature conditioning at 14°C (AB-14). Sample lanes were loaded with 10 µl of 4 mg/ml total protein and the gel was 10% acrylamide (w/v), 0.06% bis-acrylamide (w/v), pH 9.3. Protein bands identified are; N = nebulin, M = myosin, A = actin, 30kD = 30Kdalton protein. The lanes are from left to right – molecular weight standards (molecular weights (kDalton) as indicated), RB rigor, RB 4 days, AB-0 rigor, AB-0 4 days, AB-14 rigor and AB-14 4 days.



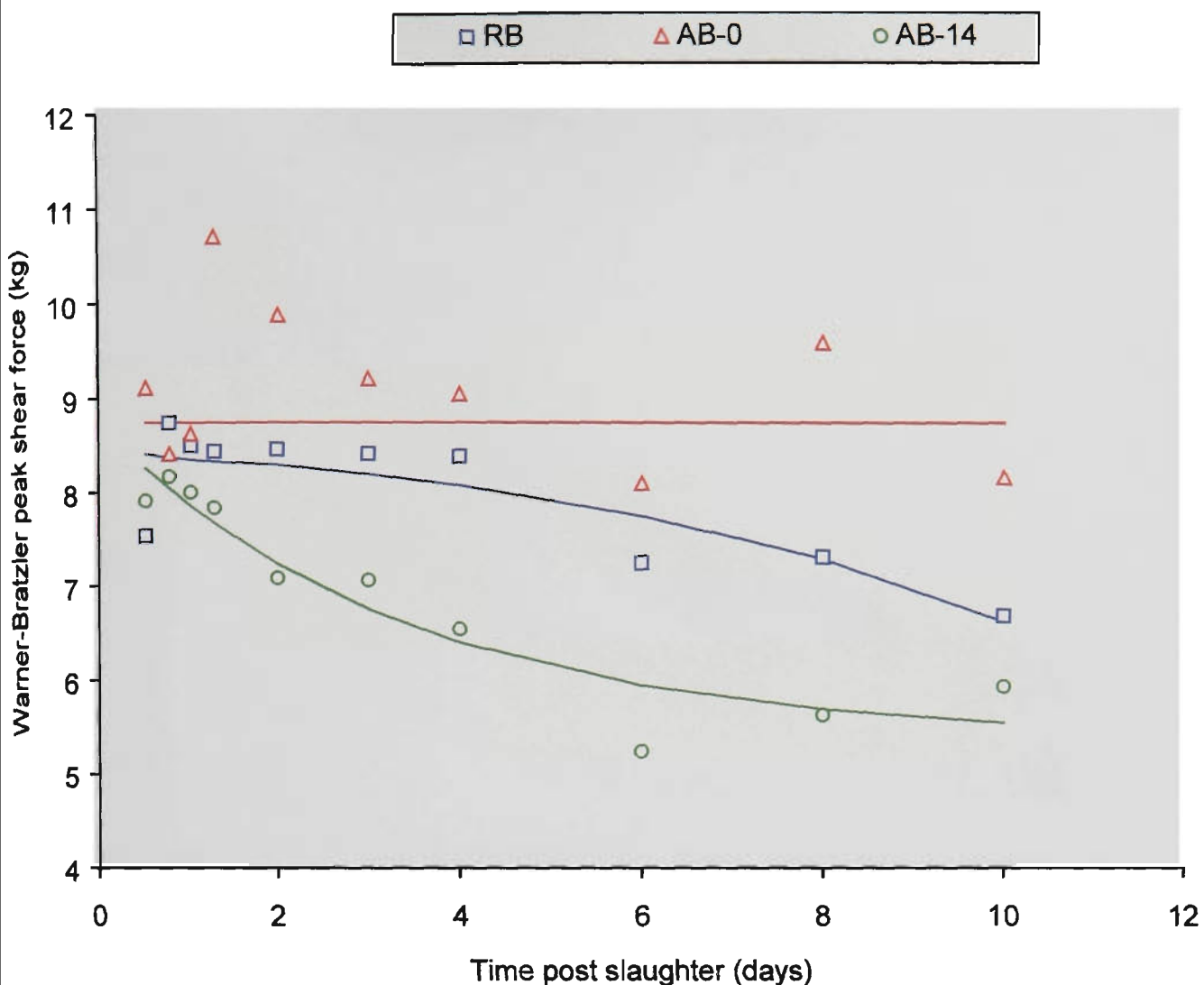


Figure 4.7 Average rate of ageing in experiment 5 of pork *M. longissimus thoracis et lumborum* after rigor boning (RB), accelerated boning and chilling at 0°C (AB-0) and accelerated boning and temperature conditioning at 14°C (AB-14).

The equations for each treatment are:

$$WBSF_{RB} = 8.79 - 0.355 e^{-0.18t} \quad (R^2 = 61.9\%, \text{RSD} = 0.44)$$

$$WBSF_{AB0} = 8.74$$

$$WBSF_{AB14} = 5.387 + 3.378 e^{-0.3t} \quad (R^2 = 87.9\%, \text{RSD} = 0.37).$$

The average SED = 0.78.

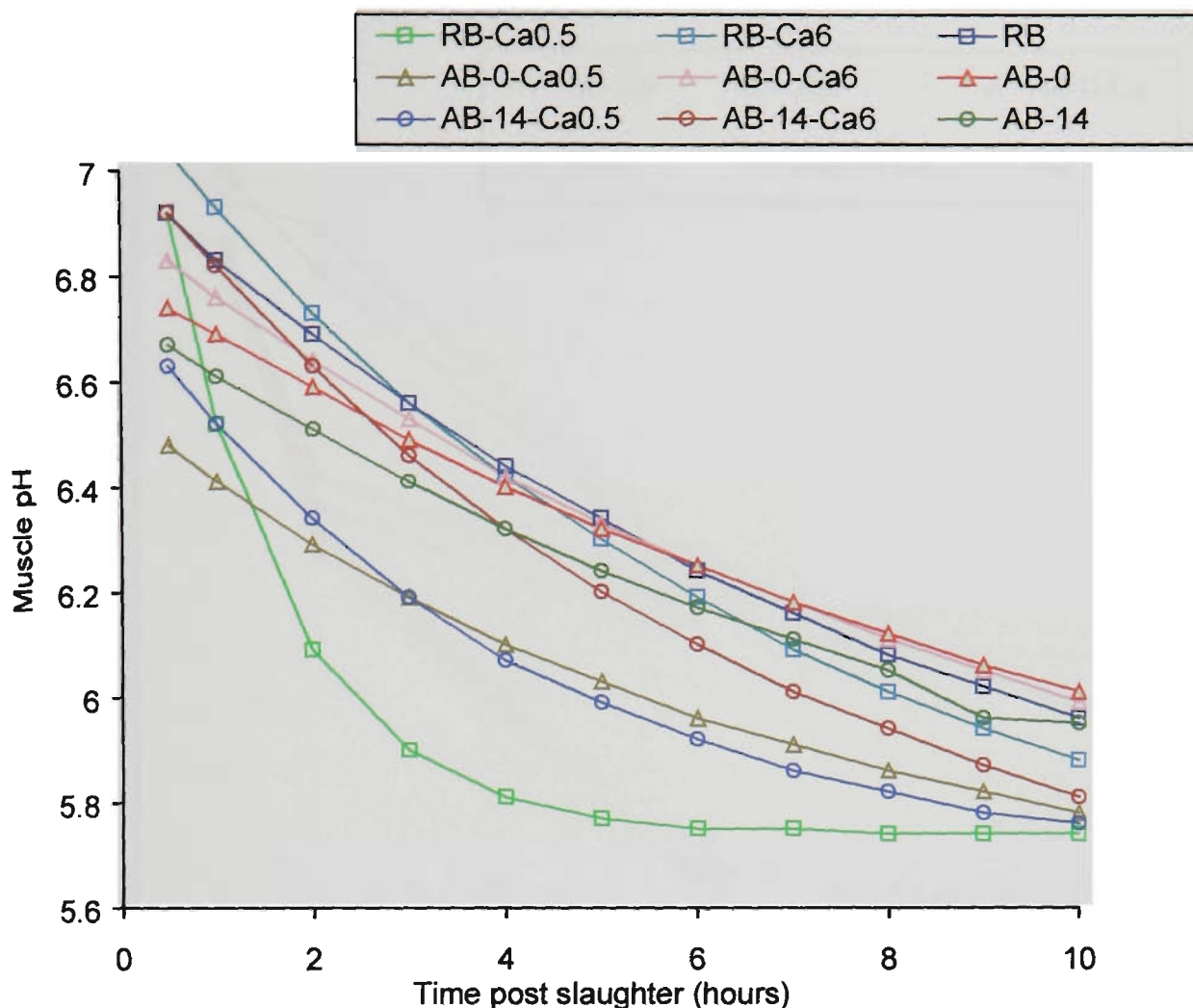


Figure 4.8 Average rate of pH decline in experiment 6 for pork *M. longissimus thoracis et lumborum* after rigor boning (RB), accelerated boning and temperature conditioning at 0 (AB-0) or 14°C (AB-14) and calcium chloride infusion at 0.5 (Ca0.5) or 6 hours (Ca6) post slaughter.

The equations for each treatment are:

$$\text{pH}_{\text{RB}} = 5.44 + 1.56 e^{-0.11t} (R^2 = 98\% \text{ RSD} = 0.07),$$

$$\text{pH}_{\text{PRB-Ca6}} = 5.51 + 1.65 e^{-0.15t} (R^2 = 97\% \text{ RSD} = 0.08),$$

$$\text{pH}_{\text{PRB-Ca0.5}} = 5.74 + 1.77 e^{-0.81t} (R^2 = 95\% \text{ RSD} = 0.09),$$

$$\text{pH}_{\text{AB-0}} = 5.41 + 1.39 e^{-0.08t} (R^2 = 97\% \text{ RSD} = 0.06),$$

$$\text{pH}_{\text{AB-0-Ca6}} = 5.53 + 1.37 e^{-0.11t} (R^2 = 97\% \text{ RSD} = 0.07),$$

$$\text{pH}_{\text{AB-0-Ca0.5}} = 5.57 + 0.98 e^{-0.15t} (R^2 = 92\% \text{ RSD} = 0.26),$$

$$\text{pH}_{\text{AB-14}} = 5.48 + 1.25 e^{-0.10t} (R^2 = 97\% \text{ RSD} = 0.06),$$

$$\text{pH}_{\text{AB-14-Ca6}} = 5.49 + 1.55 e^{-0.15t} (R^2 = 89\% \text{ RSD} = 0.16) \text{ and}$$

$$\text{pH}_{\text{AB-14-Ca0.5}} = 5.66 + 1.10 e^{-0.24t} (R^2 = 86\% \text{ RSD} = 0.12).$$

The average SED = 0.08.

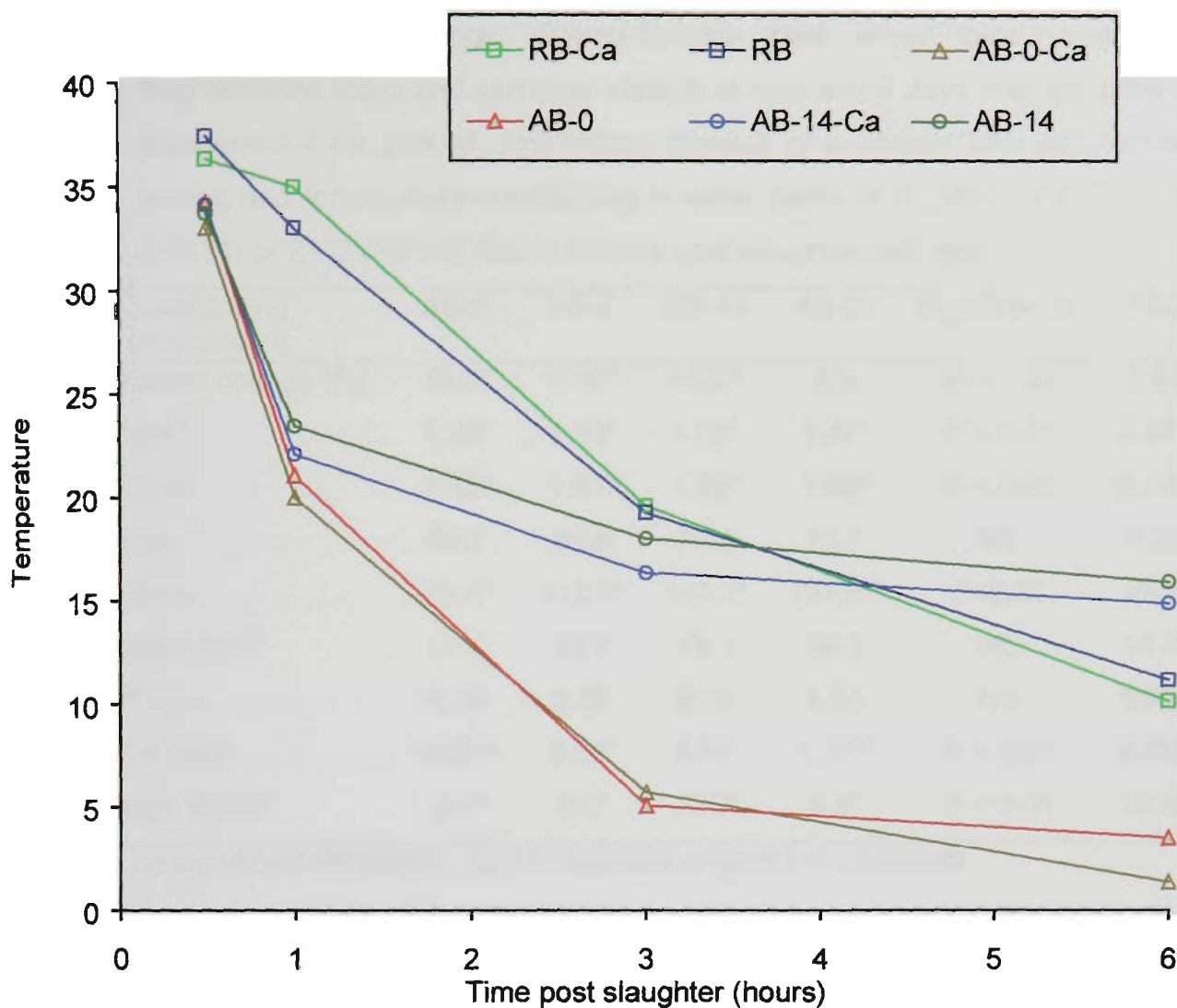


Figure 4.9 Average rate of temperature (°C) decline in experiment 6 for pork *M. longissimus thoracis et lumborum* after rigor boning (RB), accelerated boning and temperature conditioning at 0 (AB-0) or 14°C (AB-14) and calcium chloride infusion.

The average SED = 0.87.

Table 4.1 Dimensional change, Warner-Bratzler peak shear force, myofibrillar fragmentation index and sarcomere length at rigor and 4 days post slaughter in experiment 4 for pork *M. longissimus thoracis et lumborum* after accelerated boning and temperature conditioning in water baths at 0 (AB-0), 7 (AB-7), 14 (AB-14) or 21°C (AB-21) from 0.5 hours post slaughter until rigor.

Conditioning temperature	AB-0	AB-7	AB-14	AB-21	Significance ¹	SED
Dimensional change (%)	15.0 ^a	11.5 ^{ab}	11.9 ^{ab}	6.5	P < 0.05	2.90
S.L. ² rigor ³	1.30 ^a	1.60 ^b	1.58 ^b	1.47 ^b	P < 0.01	0.077
S.L. 4 days	1.45 ^a	1.67 ^b	1.69 ^b	1.65 ^b	P < 0.01	0.049
MFI ⁴ rigor	60.7	66.8	74.4	73.7	NS	8.20
MFI 4 days	74.4 ^a	113.7 ^b	145.1 ^c	131.8 ^{bc}	P<0.01	16.72
% increase MFI ⁵	17.4	38.1	46.1	35.3	NS	14.33
WBSF ⁶ rigor	9.94	9.35	9.31	8.08	NS	0.933
WBSF 4 days	10.51 ^a	8.08 ^b	6.55 ^c	7.21 ^{bc}	P < 0.01	0.597
% change WBSF	-9.4 ^a	8.0 ^a	29.7 ^b	5.8 ^a	P < 0.01	10.49

¹NS = no significant difference, SED = standard error of the difference

²S.L. = sarcomere length (μm)

³rigor = rigor, 60 minutes after pH < 5.8 reached,

⁴MFI = myofibrillar fragmentation index,

⁵% increase MFI = percentage change in MFI from rigor to 4 days post slaughter

⁶WBSF = Warner-Bratzler peak shear force (kg),

^{abc} within rows, means with different superscripts differ significantly (P<0.05)

Table 4.2 Total cathepsin B, B+L and D in experiment 4 for pork *M. longissimus thoracis et lumborum* after accelerated boning and temperature conditioning in water baths at 0 (AB-0), 7 (AB-7), 14 (AB-14) or 21°C (AB-21) from 0.5 hours post slaughter until rigor.

Conditioning Temperature	AB-0	AB-7	AB-14	AB-21	Significance	SED
Cathepsin B ¹	4.054 ^a	3.956 ^a	4.136 ^a	3.345 ^b	P < 0.05	0.261
Cathepsin B+L ¹	11.73	13.86	14.58	10.58	NS	1.781
Cathepsin D ²	0.198	0.212	0.184	0.183	NS	0.0123

^{ab} within rows, means with different superscript differ significantly (P<0.05), NS = no significant differences, SED = standard error of the difference

¹Cathepsin B and B+L activity = 1 unit of enzyme releases 1 µmol of product per minute.

²Cathepsin D 1 unit of specific activity = an increase of 0.1 absorbance units at 750nm per 60 minutes of incubation at 37°C.

Table 4.3 Meat quality at rigor and 4 days post slaughter in experiment 4 of pork *M. longissimus thoracis et lumborum* after accelerated boning and temperature conditioning in water baths at 0 (AB-0), 7 (AB-7), 14 (AB-14) or 21°C (AB-21) from 0.5 hours post slaughter until rigor.

Conditioning temperature	AB-0	AB-7	AB-14	AB-21	Significance ¹	SED
Rigor²						
pH	5.77	5.79	5.79	5.76	NS	0.044
drip loss (%) ³	1.35 ^a	0.88 ^b	0.71 ^b	1.05 ^a	P < 0.01	0.447
	(3.94)	(2.61)	(2.21)	(2.95)		
cooking loss (%)	33.19 ^a	29.87 ^{bc}	30.48 ^c	27.98 ^b	P < 0.01	1.124
L*	42.4	43.5	44.4	44.5	NS	0.861
a*	4.29	4.46	4.01	3.88	NS	0.232
b*	1.59 ^a	2.30 ^b	2.37 ^b	2.11 ^b	P < 0.001	0.174
4 days						
pH	5.50	5.49	5.53	5.51	NS	0.028
L*	47.54 ^a	49.14 ^b	50.69 ^c	50.14 ^c	P < 0.001	0.496
a*	4.43	4.33	4.54	4.55	NS	0.321
b*	2.28 ^a	2.79 ^{ab}	3.42 ^c	3.25 ^{bc}	P < 0.01	0.284
cooking loss (%)	36.10	36.54	36.72	37.16	NS	0.568
Purge (%)	6.27 ^a	4.83 ^b	3.50 ^b	3.48 ^b	P < 0.01	0.857

^{abc} within rows, means with different superscript differ significantly (P<0.05),

¹NS = no significant differences, SED = standard error of the difference

²rigor = 60 minutes post rigor as determined by pH < 5.8

³Drip loss means after log transformation, means before log transformation in brackets below

Table 4.4 Myofibrillar fragmentation index and sarcomere length measurements at rigor and 4 days post slaughter in experiment 5 of pork *M. longissimus thoracis et lumborum* after rigor boning (RB), accelerated boning and chilling at 0°C (AB-0) and accelerated boning and temperature conditioning at 14°C (AB-14).

	RB ¹	AB-0 ¹	AB-14 ¹	Significance ²	SED
SL ³ rigor ⁴	1.49	1.49	1.56	NS	0.097
SL 4 days	1.66	1.68	1.75	NS	0.054
MFI ⁵ rigor	58.8	71.6	89.6	NS	11.93
MFI 4 days	104.8 ^b	73.4 ^a	123.1 ^b	P<0.01	12.49
% increase in MFI ⁶	36.1	-3.4	18.7	NS	20.83

¹RB= post rigor boning; AB-0 = accelerated boning within 0.5 hours post slaughter and held at 0°C in an ice water bath until rigor mortis; AB-14 = accelerated boning within 0.5 hours post slaughter and held in a 14°C water bath until rigor mortis.

²NS = no significant differences. SED = standard error of the difference

³SL = sarcomere length (μm),

⁴rigor = 60 minutes post rigor as determined by pH < 5.8

⁵MFI = myofibrillar fragmentation index,

⁶% increase in MFI = percentage increase in MFI from rigor to 4 days post slaughter

Table 4.5 Warner-Bratzler peak shear force (kg) at each time post slaughter in experiment 5 of pork *M. longissimus thoracis et lumborum* after rigor boning (RB), accelerated boning and chilling at 0°C (AB-0) and accelerated boning and temperature conditioning at 14°C (AB-14).

Ageing time	RB ¹	AB-0 ¹	AB-14 ¹	Significance ²	SED
Rigor ³	7.37	8.48	7.41	NS	1.080
0.75 day	8.39	6.71	7.73	NS	1.075
1 day	8.43	8.47	8.21	NS	0.861
1.25 day	7.82 ^a	10.56 ^b	8.59 ^a	P<0.01	0.642
2 days	8.21 ^a	9.99 ^b	7.21 ^a	P<0.01	0.707
3 days	8.38 ^{ab}	8.97 ^b	7.32 ^a	P<0.05	0.591
4 days	8.09 ^b	9.19 ^b	6.68 ^a	P<0.01	0.611
6 days	7.44 ^b	7.80 ^b	5.36 ^a	P<0.001	0.727
8 days	6.98 ^a	9.55 ^b	6.00 ^a	P<0.01	0.739
10 days	6.74	7.65	6.39	NS	0.595
% change WBSF ⁴	-13.5	-15.6	2.9	NS	19.03

^{ab} within rows, means with different superscripts differ significantly (P<0.05),

¹RB= rigor boning; AB-0 = accelerated boning within 0.5 hours post slaughter and held at 0°C in an ice water bath until rigor mortis; AB-14 = accelerated boning within 0.5 hours post slaughter and held in a 14°C water bath until rigor mortis.

²NS = no significant differences. SED = standard error of the difference

³rigor = 60 minutes post rigor as determined by pH < 5.8

⁴% change in WBSF = percentage change in WBSF from rigor to 4 days post slaughter

Table 4.6 Meat quality at rigor and 4 days post slaughter in experiment 5 of pork *M. longissimus thoracis et lumborum* after rigor boning (RB), accelerated boning and chilling at 0°C (AB-0) and accelerated boning and temperature conditioning at 14°C (AB-14).

	RB ¹	AB-0 ¹	AB-14 ¹	Significance ²	SED ²
<u>Rigor³</u>					
pH	5.77 ^a	6.24 ^b	5.76 ^a	P < 0.01	0.067
Temperature (°C)	9.41 ^b	7.39 ^c	13.35 ^a	P < 0.001	0.725
L*	43.08 ^b	39.94 ^a	44.04 ^b	P<0.05	1.546
a*	3.86	3.66	4.33	NS	0.293
b*	1.89	2.10	2.61	NS	0.368
drip loss (%)	2.75 ^b	4.61 ^c	1.25 ^a	P<0.01	0.708
<u>4 days</u>					
pH	5.56 ^b	5.48 ^a	5.48 ^a	P<0.05	0.03
L*	49.82 ^a	49.33 ^a	51.50 ^b	P<0.05	0.506
a*	4.76	4.91	5.53	NS	0.686
b*	3.58	3.13	4.27	NS	0.564

^{abc} within rows, means with different superscripts differ significantly (P<0.05),

¹RB= rigor boning; AB-0 = accelerated boning within 0.5 hours post slaughter and held at 0°C in an ice water bath until rigor mortis; AB-14 = accelerated boning within 0.5 hours post slaughter and held in a 14°C water bath until rigor mortis.

²NS = no significant differences, SED = standard error of the difference

³rigor = 60 minutes post rigor as determined by pH < 5.8

Table 4.7 Cooking loss at rigor, 18, 24, 30 hours, 2, 3, 4, 6, 8 and 10 days post slaughter and purge at 4, 6, 8 and 10 days post slaughter in experiment 5 of pork *M. longissimus thoracis et lumborum* after rigor boning (RB), accelerated boning and chilling at 0°C (AB-0) and accelerated boning and temperature conditioning at 14°C (AB-14).

Time post slaughter	RB ¹	AB-0 ¹	AB-14 ¹	Significance	SED
Cooking loss (%)					
Rigor ²	28.46	28.35	27.59	NS	1.521
18 hours	32.25	30.29	29.5	NS	1.535
1 day	33.49	33.23	31.90	NS	0.974
30 hours	32.00	34.30	32.53	NS	1.470
2 days	31.31	33.73	32.34	NS	2.060
3 days	32.14 ^a	34.82 ^b	32.41 ^a	P<0.05	0.893
4 days	34.41	33.43	34.15	NS	0.513
6 days	34.83	34.28	34.78	NS	0.798
8 days	35.01	34.21	34.58	NS	1.154
10 days	33.99	34.56	35.21	NS	1.028
Purge (%)					
4 days	2.25	3.45	1.84	NS	0.953
6 days	3.86	4.95	2.37	NS	1.363
8 days	3.94 ^b	4.27 ^b	2.30 ^a	P<0.05	0.673
10 days	5.85 ^b	4.86 ^b	2.92 ^a	P<0.05	1.061

^{ab} within rows, means with different superscripts differ significantly (P<0.05), NS = no significant differences, SED = standard error of the difference

¹RB= rigor boning; AB-0 = accelerated boning within 0.5 hours post slaughter and held at 0°C in an ice water bath until rigor mortis; AB-14 = accelerated boning within 0.5 hours post slaughter and held in a 14°C water bath until rigor mortis.

²rigor = 60 minutes post rigor as determined by pH < 5.8

Table 4.8 Sarcomere length, dimensional change and myofibrillar fragmentation index measurements at rigor and 4 days post slaughter in experiment 6 for pork *M. longissimus thoracis et lumborum* after rigor boning (RB), accelerated boning and temperature conditioning at 0 (AB-0) or 14°C (AB-14) and calcium chloride infusion (average for infusion at 0.5 and 6 hours post slaughter).

Processing (Pr) ¹	RB		AB-0		AB-14		Significance ³			
	control	calcium	control	calcium	control	calcium	Pr	C	Pr x C	SED
Calcium (C) ²										
dimensional change (%)	10.0	9.0	11.9	9.3	8.2	9.0	NS	NS	NS	0.89
SL ⁴ rigor ⁵	1.66	1.51	1.40	1.45	1.52	1.54	P < 0.05	NS	NS	0.040
SL - 4 days	1.67	1.62	1.65	1.52	1.72	1.62	NS	P < 0.05	NS	0.066
MFI ⁶ rigor	45.5	50.8	57.2	55.4	58.5	46.3	NS	NS	NS	4.89
MFI 4 days	61.0	85.5	69.5	62.3	101.3	64.5	NS	NS	P < 0.05	6.89

¹RB = boning of LTL muscle at 60 minutes after rigor, AB-0 = boning of LTL muscle at 0.5 hours post slaughter and held at 0°C until rigor, AB-14 = boning of LTL muscle at 0.5 hours post slaughter and held at 14°C until rigor.

²control = no calcium chloride infusion, calcium = average for samples infused with calcium chloride (0.3M) at 0.5 and 6 hours post slaughter (10% by weight)

³NS = no significant differences, SED = standard error of the difference

⁴SL = sarcomere length (µm),

⁵rigor = 60 minutes post rigor as determined by pH < 5.8, 4 days = 4 days post slaughter

⁶MFI = myofibrillar fragmentation index,

Table 4.9 Warner-Bratzler peak shear force (kg) at rigor, 1, 2, 4 and 6 days post slaughter in experiment 6 for pork *M. longissimus thoracis et lumborum* after rigor boning (RB), accelerated boning and temperature conditioning at 0 (AB-0) or 14°C (AB-14) and calcium chloride infusion (average for infusion at 0.5 and 6 hours post slaughter).

Processing (Pr) ¹	RB		AB-0		AB-14		Significance ³		
	control	calcium	control	calcium	Control	calcium	C	C x T	SED
Calcium (C) ²									
rigor ⁴	8.21	6.76	9.72	7.33	8.38	7.09	P < 0.001	NS	0.423
1 day	7.65	5.50	7.44	6.00	6.63	5.90	P < 0.001	NS	0.396
2 days	7.10	4.86	7.32	6.16	6.99	5.76	P < 0.001	NS	0.329
4 days	5.88	4.25	6.63	4.53	5.35	4.05	P < 0.001	NS	0.365
6 days	6.60	4.58	6.76	4.73	5.73	4.66	P < 0.001	P < 0.05	0.259
% change ⁵	26.6	34.4	29.9	35.9	33.6	42.4	NS	NS	9.41

¹RB = boning of LTL muscle at 60 minutes post rigor, AB-0 = boning of LTL muscle at 0.5 hours post slaughter and held at 0°C until rigor, AB-14 = boning of LTL muscle at 0.5 hours post slaughter and held at 14°C until rigor.

²control = no calcium chloride infusion, calcium = average for samples infused with calcium chloride (0.3M) at 0.5 and 6 hours post slaughter (10% by weight),

³NS = no significant differences, SED = standard error of the difference, T = tie of infusion, either 30 minutes or 6 hours post slaughter, No processing, processing x calcium or processing x calcium x time effects were observed

⁴rigor = shear force measurements performed at 60 minutes post rigor (as determined by pH < 5.8),

⁵% change = percentage change in WBSF between rigor and 4 days post slaughter

Table 4.10 Meat quality at rigor, 1 and 4 days post slaughter in experiment 6 for pork *M. longissimus thoracis et lumborum* after rigor boning (RB), accelerated boning and temperature conditioning at 0 (AB-0) or 14°C (AB-14) and calcium chloride infusion (average for infusion at 0.5 and 6 hours post slaughter).

Processing (Pr) ¹	RB		AB-0		AB-14		Significance ³			
	control	calcium	control	calcium	Control	calcium	Pr	C	Pr x C	SED
Calcium (C) ²										
pH rigor	5.79	5.82	6.12	5.91	5.76	5.72	P < 0.001	NS	NS	0.040
Drip loss (%) rigor	3.2	6.2	4.6	7.0	1.1	6.1	P < 0.05	P < 0.001	NS	0.54
L* rigor	42.6	45.1	39.4	45.5	44.6	46.0	P < 0.05	P < 0.05	NS	0.94
a* rigor	3.72	3.48	3.72	3.56	3.68	3.84	NS	NS	NS	0.357
b* rigor	1.40	1.84	1.87	2.00	2.47	2.42	P < 0.01	NS	NS	0.272
pH 1 day	5.4	5.6	5.5	5.5	5.5	5.6	NS	NS	NS	0.04
L* 1 day	49.5	48.5	49.3	47.7	51.7	49.7	P < 0.05	NS	NS	0.62
a* 1 day	4.62	4.34	4.25	4.06	5.15	4.20	NS	NS	NS	0.465
b* 1 day	2.45	2.56	2.62	2.32	3.34	2.71	NS	NS	NS	0.351
pH 4 days	5.5	5.6	5.5	5.6	5.5	5.6	NS	P < 0.001	NS	0.03
L* 4 days	48.6	49.6	51.0	45.6	50.3	48.7	NS	P < 0.05	P < 0.05	0.61
a* 4 day	4.59	4.14	3.73	3.98	4.40	4.29	P < 0.01	NS	NS	0.252
b* 4 day	2.99	2.98	2.42	2.52	3.11	3.06	NS	NS	NS	0.348

¹RB = boning of LTL muscle at 60 minutes post rigor, AB-0 = boning of LTL muscle at 0.5 hours post slaughter and held at 0°C until rigor, AB-14 = boning of LTL muscle at 0.5 hours post slaughter and held at 14°C until rigor.²control = no calcium chloride infusion, calcium = average for samples infused with calcium chloride (0.3M) at 0.5 and 6 hours post slaughter (10% by weight),
³NS = no significant differences, SED = standard error of the difference

Table 4.11 Cooking loss at rigor, 1, 2, 4 and 6 days post slaughter and purge at 4 and 6 days post slaughter in experiment 6 for porkM. *longissimus thoracis et lumborum* after rigor boning (RB), accelerated boning and temperature conditioning at 0 (AB-0) or 14°C (AB-14) and calcium chloride infusion (average for infusion at 0.5 and 6 hours post slaughter).

Processing (Pr) ¹	RB		AB-0		AB-14		Significance ³				
	control	calcium	control	calcium	control	Calcium	Pr	C	PrxC	PrxCaxT	SED
Calcium (C) ²											
Cooking loss (%)											
rigor	29.5	34.0	29.2	35.0	26.5	34.4	NS	P < 0.001	NS	NS	0.66
1 day	35.1	35.5	35.5	35.8	31.8	35.6	NS	P < 0.01	P < 0.01	NS	0.75
2 day	35.4	34.3	35.1	35.5	31.7	34.5	P < 0.001	NS	P < 0.05	NS	0.68
4 day	33.9	33.5	35.9	34.6	35.0	33.9	NS	NS	NS	NS	0.47
6 day	35.1	34.5	35.5	34.4	35.4	34.4	NS	P < 0.05	NS	NS	0.66
Purge (%)											
4 day	4.8	11.6	4.7	8.3	2.0	10.4	P < 0.05	P < 0.001	P < 0.01	P < 0.001	1.20
6 day	4.6	10.4	5.1	9.2	1.9	11.1	NS	P < 0.001	NS	P < 0.01	1.43

¹RB = boning of LTL muscle at rigor, 60 minutes post rigor as determined by pH < 5.8, AB-0 = boning of LTL muscle at 0.5 hours post slaughter and held at 0°C until rigor, AB-14 = boning of LTL muscle at 0.5 hours post slaughter and held at 14°C until rigor.

²control = no calcium chloride infusion, calcium = average for samples infused with calcium chloride (0.3M) at 0.5 and 6 hours post slaughter (10% by weight),

³NS = no significant differences, SED = standard error of the difference, PrxCaxT = processing x calcium x time interaction effects

5. RESPONSE OF THE RATE OF AGEING TO THE RATE OF pH AND TEMPERATURE DECLINE FOR PORK AND LAMB.

5.1 INTRODUCTION

Earlier experiments have found different ageing curves for rigor boned pork (chapters 3 and 4). In both these experiments, the same genetics, transport, lairage, handling, stunning, slaughtering and chilling conditions were employed. Due to the different rates of pH and temperature decline between the two experiments, it is postulated that this was the cause of the variation in rate of ageing.

Furthermore, variations in both the rate of pH decline and the rate of ageing have been reported in the literature for lamb and pork (Koohmaraie *et al.*, 1991; Judge *et al.*, 1989; Dransfield *et al.*, 1980-81; Harrison *et al.*, 1970). Lamb has been reported to take 6 to 12 hours to reach the onset of rigor mortis while pork carcasses have been reported to reach this stage within ¼ to 3 hours post slaughter (Judge *et al.*, 1989). In contrast to these fast rates of rigor onset for pork in the literature, in the various experiments conducted in this research pork has taken from 7 to 12 hours to reach rigor. Pork has been reported to completely age within 4-6 days post slaughter (Dransfield *et al.*, 1980-81; chapter 3) which contrasts with longer ageing periods observed in chapter 4 and those reported by Harrison *et al.* (1970) and Buchter and Zeuthen (1971). In contrast, lamb has been reported to require 8-10 days for complete ageing to occur (Dransfield *et al.*, 1980-81). Variations in rate of pH decline may be a contributing factor to the variations in rate of ageing between species and studies (Koohmaraie *et al.*, 1991).

The rate of rigor development is believed to influence tenderness and subsequent ageing due to a number of factors. With an increase in the rate of rigor development, the tenderising enzymes are activated earlier and therefore at higher temperatures. The earlier activation of these enzymes is due to the early release of calcium ions from the sarcoplasmic reticulum activating the calpain enzymes (Dransfield, 1994; Geesink, 1993; Ouali and Talmont, 1990) and the earlier release of the cathepsins from the lysosomes as a result of the lower pH (Etherington, 1989). In addition to

changes in enzymatic activity, variation in pH and temperature decline can alter muscle structure through shortening and protein denaturation.

One factor that will influence the rate of ageing is the level of protein denaturation that occurs during rigor development. Protein denaturation is strongly influenced by the rate of pH and temperature decline with rapid rates of pH decline being associated with protein denaturation, resulting in PSE (D'Souza *et al.*, 1998; Briskey and Wismer-Pedersen, 1961). This rapid pH decline of PSE muscle results in a high level of myofibrillar and sarcoplasmic protein denaturation, including denaturation of such proteins as myosin and possibly the glycolytic and proteolytic enzymes (Wismer-Pedersen, 1959). The denaturation of the tenderising enzymes can help to explain the lack of improvement in tenderness with storage post slaughter of PSE pork. Thus the ideal rate of pH decline must be determined in order to prevent denaturation thereby allowing improvements in tenderness to occur with post slaughter storage.

The most widely reported influence of pH and temperature decline on tenderness has been due to the impact of cold toughening (Moller and Jensen, 1993; Kauffman and Marsh, 1987; Dutson and Pearson, 1985; Hamm, 1981; Marsh and Leet, 1966). Cold toughening involves both cold shortening and a reduction in proteolytic activity due to the rapid fall in muscle temperature. Cold shortening occurs when pH decline has been slow with a fast muscle temperature decline resulting in muscle temperatures below 10°C before rigor onset (Hamm, 1981). The prevention of shortening pre-rigor improves meat tenderness (Lochner *et al.*, 1980). Studies by Lochner *et al.* (1980) in beef found that loin steaks with the fastest cooling rate were found to be the least tender and have the shortest sarcomere length. In the study by Lochner *et al.* (1980) tenderness was found to be highly dependent and almost linearly related to the muscle temperature attained at 2 hours post slaughter which suggests that temperature effects on tenderness are not just cold shortening.

Many studies of severe cold shortening have involved muscles undergoing accelerated boning (Koochmaraie *et al.*, 1996; Iversen *et al.*, 1995; Swatland, 1995, Dransfield and Lockyer, 1985; Marsh and Leet, 1966). Conventionally boned muscles appear to be less susceptible to cold shortening than muscles following accelerated boning due to the physical restraint provided by the carcass and the slower temperature decline (Polidori *et al.*, 1996). The slower temperature decline

can be attributed to the primals not being separated and thus chilling more slowly and the skin and subcutaneous fat providing thermal insulation. However, if chilling conditions are severe enough cold shortening can still occur in conventionally processed muscles. Furthermore, the extent of cold shortening that can occur has been reported to be influenced by species with lamb being more susceptible to cold shortening than pork (Marsh, 1981). This increased susceptibility to cold shortening of lamb is due to the higher number of mitochondria present in the muscles relative to pork due to more red fibres that are more susceptible to cold shortening than white fibres (Marsh, 1981). The higher mitochondria content allows a greater release of calcium ions thereby promoting contraction due to the lower affinity of mitochondria for calcium at reduced temperatures.

Some of the impact of chilling conditions can be eliminated by the method of carcass suspension. Suspending carcasses from the pelvic bone (instead of the Achilles tendon) reduces shortening in the LTL of conventionally processed muscle (Moller *et al.*, 1987). This is called tenderstretch and has been implemented in systems to produce quality beef in the UK and Australia. In pork, pelvic suspension after blast chilling (-18°C for 65 minutes, subsequently chilled at 2-4°C) relative to muscles suspended in the Achilles tendon improves *longissimus* tenderness (Moller *et al.*, 1987). Furthermore, improved tenderness and longer sarcomere length were seen by Moller and Vestergaard (1986) with pelvic suspension when pork sides were chilled at -28 to -22°C for 65 minutes then at 2-4°C. Pelvic suspension has also been reported to improve tenderness in beef carcasses (Hostetler *et al.*, 1975; Herring *et al.*, 1965). These improvements in tenderness are believed to be due to a reduction in sarcomere length shortening and stretching of the muscle.

Several processes can influence the rate of pH decline post slaughter. One of the most widely used methods to speed up the rate of pH decline, used extensively in the beef and lamb industry, is electrical stimulation. Electrical stimulation of beef carcasses within the first hour post slaughter using low or high voltage accelerates the rate of pH decline relative to carcasses that are not electrically stimulated (Kastner *et al.*, 1993; Takahashi *et al.*, 1984; Bowles *et al.*, 1983). Different pH decline rates are seen when different current, voltage and time of application are used (Chrystall and Devine, 1978). Care must be taken to avoid a rapid pH decline without modifying temperature decline due to the potential to induce protein

denaturation. For example, (Unruh *et al.*, 1986) found negative changes in tenderness and juiciness occurred with rapid pH decline and slow temperature decline thereby increasing toughness. Thus an optimal rate of pH decline during rigor development is desirable for a given species and chilling rate.

Electrical stimulation was originally developed to prevent cold shortening. Electrical stimulation prevents cold shortening by increasing the rate of pH decline, speeding up the rigor process. This induces the reduction of ATP and other high energy phosphates during rigor development, further assisting in preventing cold shortening (Sorinmade *et al.*, 1982; Aalhus *et al.*, 1994). In addition to accelerating the onset of rigor, electrical stimulation causes proteolytic induced tenderisation to begin at higher temperatures and induces physical disruption of the myofibrils (Savell *et al.*, 1978).

Improvements in pork tenderness early post rigor have been seen after electrical stimulation (Polidori *et al.*, 1996; Savell, 1979). High voltage and low voltage electrical stimulation resulted in an improvement in pork tenderness of 28 and 17-18% respectively compared to non stimulated carcasses (Taylor and Martoccia, 1995). However, the impact of electrical stimulation on tenderness has been observed to decline with post slaughter storage with the ultimate tenderness being the same for both stimulated and non-stimulated muscles (Dransfield, 1994).

Another factor that alters the rate of pH decline is the method of stunning employed with either electrical or carbon dioxide stunning being the preferred methods in the pork industry (Barton-Gade, 1993). Electrical stunning has been shown to increase the rate of glycogen metabolism post slaughter in a manner similar to electrical stimulation (Barton-Gade, 1993) particularly if stunning provokes excessive muscular contraction (Barton-Gade, 1980). Channon *et al.* (1997) found that a faster rate of pH decline occurred in the *M. longissimus thoracis et lumborum* (LTL) muscle of pigs stunned electrically using head-to heart tongs relative to pigs stunned in 90% carbon dioxide in air although the ultimate pH was the same. Electrically stunned pigs also had more tender meat at 1 day post slaughter than the carbon dioxide stunned pigs. Due to the increasing use of carbon dioxide stunning in the Australian pig industry, the need exists for a greater understanding of the influence of carbon dioxide stunning on the rate of pH decline and rate of ageing.

Differences in pH decline due to the method of stunning can be attributed to the differences in stunning response. Pigs electrically stunned become unconscious due to an induced epileptic seizure (Gregory, 1985). The seizure results in a release of neurotransmitters, increasing muscular glycolysis thereby influencing the rate of rigor development (Gregory, 1985). On the other hand, carbon dioxide stunning results in anaesthesia and little muscular contraction (Lomholt, 1980). Glycolysis is not stimulated with carbon dioxide stunning although muscle pH may be slightly lowered due to the increased concentration of carbon dioxide in the blood and muscle (Channon *et al.*, 1997).

Due to the variations seen in pH decline rates and the impact this may have on the rate of ageing, several experiments were designed to determine if:

- The rate of pH decline can alter the rate of change in tenderness with post slaughter storage
- A fast rate of pH decline with a slow decline in muscle temperature will result in protein denaturation thereby preventing improvements in tenderness with post slaughter storage
- A slow rate of pH decline with a fast decline in muscle temperature will result in cold toughening thereby preventing improvements in tenderness with post slaughter storage
- An intermediate rate of pH and temperature decline will prevent protein denaturation and cold toughening and promote proteolytic activity thereby resulting in improvements in tenderness with post slaughter storage.

In experiment 7 electrical stimulation and different stunning methods were used to induce different rates of pH decline. In addition, the impact of suspension methods was investigated to determine if this could overcome cold shortening. In experiment 8, the impact of different chilling temperatures combined with different rates of pH decline on the rates of ageing was investigated. Finally in experiment 9, lamb was investigated to determine whether the response to the rate of pH decline to the rate of ageing was altered by the different properties of white and red muscle fibre types in the two species.

5.2 METHODOLOGY

5.2.1 Experiment 7: Influence of rate of pH decline on the rate of ageing of pork

Twenty-four male finisher pigs were slaughtered over three slaughter days and randomly allocated to a 2 x 2 factorial combination of stunning method and electrical stimulation using conditions outlined in Chapter 2. The stunning treatments were electrical stunning using head to heart tongs (E) or carbon dioxide stunning (C). The stimulation treatments were no electrical stimulation (N) or low voltage stimulation (S). A split plot design was used for the suspension treatments following carcass splitting with either pelvic (P) or Achilles (A) suspension used for each side. Thus the treatments were:

1. Stunned in 90% CO₂ in air with an exposure time of 1.8 minutes and no electrical stimulation (CN)
2. Stunned in 90% CO₂ in air, low voltage electrically stimulated at 4 minutes post slaughter for 15 seconds as described in Chapter Two (CS)
3. Electrically stunned - head to heart (1.3 amps, 4 sec) and no electrical stimulation (EN)
4. Electrically stunned - head to heart (1.3 amps, 4 sec) and electrically stimulated at 4 minutes post slaughter for 15 seconds (ES).

The carcasses were split and the sides subjected to pelvic suspension (P) or Achilles suspension (A). At 0.5 hours post slaughter, carcasses were chilled at 2°C resulting in a temperature decline rate constant of approximately 0.3. The pH and temperature of the LTL was monitored at 40 minutes, 1 hour then every hour until a pH of less than 5.8 was obtained. The pH and temperature were measured at the 5th thoracic vertebra, 13th thoracic vertebra and 5th lumbar vertebra. Rigor was defined as one hour after the pH was less than 5.8 at all 3 sites. The LTL was removed at rigor and split into eight 150 g samples. Seven 150 g samples were randomly allocated to an ageing period, vacuum packaged and stored at 2°C until further testing and the remaining sample was used for WBSF, meat quality and drip loss determination.

5.2.1.1 Meat quality measurements

At rigor, the pH, colour (CIE - L*, a*, b*) and drip loss were determined at the 13th rib and a sample was cooked for cooking loss and WBSF. Cooking loss and WBSF were again determined at 1, 2, 4, 6, 8, and 10 days post slaughter. Purge was determined at 4 and 10 days post slaughter. All methods are outlined in Chapter Two.

5.2.1.2 Biochemical measurements

At 1, 3 and 5 hours post slaughter and at rigor, 2g muscle samples were collected and immediately processed for protein solubility using method 2 outlined in Chapter Two.

At rigor and at 4 days post slaughter, 50 g muscle samples were collected, frozen in liquid nitrogen and stored at -80°C for subsequent measurement of sarcomere length, myofibrillar fragmentation index (MFI) and also subjected to SDS-PAGE to assess protein degradation. All methods are outlined in Chapter Two.

5.2.2 Experiment 8: Influence of rate of pH and temperature decline on the rate of ageing of pork

Twenty-four male finisher pigs were slaughtered over three slaughter days and randomly allocated to one of four treatments designed to alter the rate of pH decline. The stunning treatments were either electrical stunning using head to heart tongs (E) or carbon dioxide stunning (C). The electrical stimulation treatments were no stimulation (N), stimulation for 15 seconds (S), or stimulation for 60 seconds (L). A split plot design was used for the chilling treatment with sides being chilled at 2 or 14°C. Thus the treatments were:

1. Electrically stunned - head to heart (1.3 amps, 4 sec) and no electrical stimulation (EN),
2. Stunned in 90% carbon dioxide in air as previously reported (Chapter Two) and no electrical stimulation (CN),
3. Stunned in 90% carbon dioxide in air, electrically stimulated as described in Chapter Two at 4 minutes post slaughter for 15 seconds (CS),

4. Stunned in 90% carbon dioxide in air, electrically stimulated at 4 minutes post slaughter for 60 seconds (CL).

The carcasses were split, the sides were suspended by the Achilles tendon and the sides were randomly allocated to one of two chilling regimes - fast chilling (chilled at 0-2°C) or slow chilling (chilled at 14-16°C). The sides were chilled at these temperatures until rigor as defined previously, at which time the LTL muscle was removed and the cranial end was separated into six 150 g samples which were randomly allocated to an ageing period. The samples were vacuum packaged and stored at 2°C until assessment. The caudal end (700 g) was vacuum packaged and stored at 2°C for 2 days for taste panel analysis using the method outlined in Chapter Two. The sensory panel consisted of anglo-saxons with the ratio of males to females being 7:3 with 14% of the panel aged 18-24, 22% between 25 and 30, 36% between 31 and 39 and 28% aged 40 to 49 years. Fifty-eight percent of the panel reported that they eat roast pork every 3 months, 33% every month and 5% every fortnight.

5.2.2.1 Meat quality measurements

The pH and temperature of the LTL was measured at 30 minutes, 1 hour then every hour until a pH of less than 5.8 was obtained as described in Chapter Two. The measurements were made at the 5th thoracic vertebra, 13th thoracic vertebra and 5th lumbar vertebra.

At rigor, the pH, colour (CIE - L*, a*, b*), temperature and drip loss were determined at the 13th rib and the first sample was cooked for cooking loss and WBSF as described in Chapter Two. Cooking loss and WBSF were again determined at 1, 2, 4, and 8 days post slaughter and purge was determined at 4 and 8 days post slaughter.

5.2.2.2 Biochemical measurements

At rigor, 2g muscle samples were collected and immediately processed for sarcoplasmic protein solubility using method 1 outlined in Chapter Two.

At rigor and 4 days post slaughter, 50 g muscle samples were collected, frozen in liquid nitrogen and stored at -80°C for later assessment of sarcomere length, MFI and SDS-PAGE as outlined in Chapter Two.

5.2.3 Experiment 9: Influence of rate of pH decline on the rate of ageing of lamb

Twenty-four prime lambs were randomly allocated to one of two treatments designed to alter the rate of pH decline:

1. Electrically stunned as described in Chapter Two, no electrical stimulation, or
2. Electrically stunned and low voltage electrical stimulation applied for 50 seconds at 5 minutes post slaughter using the system described in Chapter Two.

The carcasses were slaughtered over three slaughter days. The carcasses were split following evisceration and the sides randomly allocated to pelvic or Achilles suspension before chilling from 1 hour post slaughter at 2°C. At rigor, the LTL muscle was removed, and split into six 80g samples. The samples were randomly allocated to an ageing period, vacuum packaged and stored at 2°C until testing.

5.2.3.1 Meat quality measurements

The pH and temperature of the LTL was measured at 40 minutes, 1 hour then every hour post slaughter until a pH of less than 5.8 was obtained. These were measured as for the previous experiment at the 5th thoracic vertebra, 13th thoracic vertebra and 5th lumbar vertebra. Rigor was defined as 60 minutes after a pH less than 5.8 occurred at all 3 sites.

At rigor, the pH and colour (CIE - L*, a*, b*) were determined at the 13th rib and a sample was cooked for cooking loss and WBSF using the methods outlined in Chapter Two. Cooking loss and WBSF were again determined at 1, 2, 4, 6, 8, and 10 days post slaughter and purge was determined at 4 and 10 days post slaughter.

5.2.3.2 Biochemical measurements

At 1, 3 and 5 hours post slaughter and at rigor, 2g muscle samples were collected and immediately processed for protein solubility using method 2 outlined in Chapter Two.

At rigor 50 g muscle samples were collected, frozen in liquid nitrogen and stored at -80°C for later assessment of sarcomere length, MFI and application of myofibrillar proteins to SDS-PAGE as outlined in Chapter Two. Ten gram samples for SDS-PAGE were also collected at 4 days post slaughter.

5.2.4 Statistics

The results from these experiments were analysed using the ANOVA function of Genstat 5 (Payne *et al.*, 1988). The data for each experiment was analysed by ANOVA using a completely randomised factorial design. The data was blocked according to animal and slaughter day. Correlation coefficients for sarcomere length, MFI and WBSF data were determined. The highest level of significant interaction is presented in the results section and only main effects are presented when no interactions occurred. Due to negligible interactions occurring, the data presented in the tables are main effects.

The rate of pH and temperature decline and rate of ageing for each treatment was determined by fitting the following equation to the data using the fit curve function of Genstat 5 (Payne *et al.*, 1988):

$$F_t = F_\infty + (F_0 - F_\infty)e^{-kt}$$

where F_t , F_0 and F_∞ are the WBSF/pH/temperature values at time t , at time zero (stunning for pH and temperature decline and rigor for WBSF) and at the completion of ageing/pH/temperature decline respectively and k is the rate constant. Individual curves were fitted to each muscle and the rate constants were analysed to determine if differences occurred between the treatments. The average curve for each treatment was determined by fitting the curve to the average values for each treatment for each time measured.

The percentage change from rigor to 4 days post slaughter was determined for WBSF and MFI using the following equations:

$$\% \text{ change WBSF} = \frac{\text{WBSF}_{\text{rigor}} - \text{WBSF}_{4\text{days}}}{\text{WBSF}_{\text{rigor}}} \times 100$$

$$\% \text{ change MFI} = \frac{\text{MFI}_{4 \text{ days}} - \text{MFI}_{\text{rigor}}}{\text{MFI}_{4 \text{ days}}} \times 100$$

5.2.4.1 Analysis grouped according to the rate of pH decline

Data was also analysed after combining animals from experiment 7 and 8 and subjected to grouping on their rate of pH decline. This gave three groups - slow ($k < 0.10$), medium ($0.11 < k < 0.40$) and fast ($k > 0.41$) and analysed according to their rates using ANOVA. The average rate of ageing, pH and temperature decline were determined for these groups. Results from the lamb carcasses in experiment 9 were grouped according to the rate of pH decline into two groups and analysed as for the pig carcasses. The two groups were slow ($k < 0.20$) and medium ($0.21 < k < 0.74$).

5.3 RESULTS

5.3.1 Experiment 7: Influence of rate of pH decline on the rate of ageing of pork

5.3.1.1 Sample description

The average live weight of the pigs slaughtered was 91.7 ± 5.32 kg. This resulted in an average carcass weight of 65.3 ± 5.87 kg with an average fat depth at the P₂ site of 18.5 ± 4.21 mm. One side could be classified as DFD (carbon dioxide stunned, no stimulation) and 6 sides could be classified as PSE ($n = 4$ – electrical stunning and stimulated, $n = 1$ – electrical stunning, no stimulation, $n = 1$ – carbon dioxide stunning, no stimulation) using the definition of PSE and DFD described by Warner *et al.* (1993).

5.3.1.2 pH and temperature decline

The rate of pH decline seen in Figure 5.1, was influenced by stimulation ($P < 0.05$) but not by stunning method or the method of suspension due to the high standard error ($P > 0.05$). The average pH for the three sites was lower for electrical

stimulation compared to non-stimulated carcasses at 40 minutes, 1, 2, 3, 4 and 5 hours post slaughter ($P < 0.001$). At 1, 2 and 3 hours post slaughter, the pH was also lower for electrical stunning relative to carbon dioxide stunning ($P < 0.05$) but the method of stunning had no effect ($P > 0.05$) on pH at 40 minutes or 5 hours post slaughter.

The time taken to reach rigor was influenced by both the method of stunning ($P < 0.05$) and the use of electrical stimulation ($P < 0.001$) (Table 5.1). Stimulated carcasses took 4 hours less than the non-stimulated carcasses to reach a pH of less than 5.8 while the carbon dioxide stunned pigs took over 1.5 hours longer to obtain this pH than the electrically stunned pigs. The method of suspension had no effect ($P > 0.05$) on the time taken to reach rigor.

The rate of temperature decline was not influenced by electrical stimulation, the method of stunning or the method of suspension. The average rate of temperature decline for all pigs fitted an exponential curve ($R^2 = 99.9\%$, $RSD = 0.29$) and can be seen in Figure 5.2.

5.3.1.3 Sarcomere length and myofibrillar fragmentation index

Sarcomere length measured at both rigor and 4 days post slaughter was longer ($P < 0.05$) in the electrically stimulated muscles relative to the non-stimulated muscles (Table 5.1). A trend towards longer sarcomere length was observed in the pelvic suspended sides relative to the Achilles tendon suspension at rigor ($P = 0.06$). An interaction between stimulation and suspension method ($P < 0.05$) was observed at rigor with the muscles that received stimulation and pelvic suspension having a longer sarcomere length than the muscles which were stimulated and suspended by the Achilles and the muscles which were not stimulated regardless of the method of suspension. At 4 days post slaughter, the pelvic suspended muscles had a longer sarcomere length than the Achilles tendon suspended muscles ($P < 0.05$). At 4 days post slaughter, the carbon dioxide stunned muscles also showed a trend towards shorter sarcomere length relative to the electrically stunned muscles ($P = 0.09$) but not at sampling ($P > 0.05$).

Electrical stimulation, the method of stunning and the method of suspension did not influence ($P > 0.05$) the myofibrillar fragmentation index at either rigor or at 4 days post slaughter (Table 5.1) but the MFI doubled between rigor and 4 days post slaughter indicating extensive ageing occurred. The percentage change in MFI from rigor to 4 days post slaughter was influenced by low voltage electrical stimulation ($P < 0.05$) with the muscles undergoing stimulation having a smaller percentage change than the non- stimulated muscles (Table 5.1).

At rigor there were no correlations between sarcomere length and WBSF ($r = -0.218$, $P > 0.05$), sarcomere length and MFI ($r = 0.069$, $P > 0.05$), or MFI and WBSF ($r = -0.069$, $P > 0.05$). At 4 days post slaughter there was a significant correlation between WBSF and sarcomere length ($r = -0.431$, $P < 0.05$) but not between MFI and sarcomere length ($r = -0.222$, $P > 0.05$) or WBSF and myofibrillar fragmentation index ($r = -0.125$, $P > 0.05$).

5.3.1.4 Sarcoplasmic and myofibrillar protein solubility

Sarcoplasmic protein solubility measured at 1, 3 and 5 hours post slaughter and rigor was not influenced ($P > 0.05$) by the method of stunning or method of stimulation (Table 5.2). There was also no differences ($P > 0.05$) in myofibrillar protein solubility at 1, 3 and 5 hours post slaughter but at rigor, the carbon dioxide stunned muscles had a lower myofibrillar protein solubility relative to the electrically stunned carcasses ($P < 0.05$) (Table 5.2).

5.3.1.5 Protein Degradation

A representative SDS-PAGE gel for the different treatments can be seen in Figure 5.3. A comparison of protein degradation in myofibril samples from rigor to 4 days post slaughter using SDS-PAGE illustrates that ageing occurred over this time period. Changes in both nebulin and titin bands occurred between rigor and 4 days post slaughter for all treatments. The nebulin band appears to have been degraded due to the disappearance of the nebulin band by 4 days post slaughter. However, not all of the nebulin band has disappeared for the muscles which received no stimulation and where stunned with carbon dioxide. The most degradation of nebulin appears to have occurred in the muscles following electrical stunning and no electrical

stimulation. Troponin T appears to have been degraded in all of the treatments and a 30 kD band has appeared for all treatments by 4 days post slaughter.

5.3.1.6 Warner-Bratzler shear force and ageing

The WBSF values measured over a 10 day ageing period can be seen in Table 5.3. WBSF was reduced ($P < 0.01$) by pelvic suspension at rigor, 1, 2 and 6 days post slaughter relative to carcasses suspended by the Achilles tendon. The WBSF was also reduced by electrical stimulation relative to non-stimulated carcasses at 1, 2 and 10 days post slaughter ($P < 0.05$) while electrical stunning reduced WBSF at 1 and 2 days post slaughter relative to those stunned with carbon dioxide ($P < 0.05$).

The rate of change in WBSF over a ten day ageing period as determined by fitting an exponential decay equation was increased by low voltage electrical stimulation compared to non-stimulated carcasses ($P < 0.05$) but was not influenced by the method of stunning or the method of suspension (Figure 5.4). The percentage change in WBSF from rigor to 4 days post slaughter was increased by stimulation ($P < 0.05$) relative to the muscles which were not stimulated (Table 5.3).

5.3.1.7 Colour, pH and drip loss

The effect of treatments on L^* , a^* , b^* , pH and drip loss at rigor and 4 days post slaughter are given in Table 5.4. At rigor, the electrically stimulated LTL muscles had a lower pH than the non-stimulated muscles at the P_2 site ($P < 0.001$) and a higher L^* value ($P < 0.05$). L^* , a^* and b^* values and pH were not influenced ($P > 0.05$) by the method of stunning or the method of suspension. Drip loss measured at rigor was increased by electrical stunning relative to carbon dioxide stunning ($P < 0.05$) but was not influenced by electrical stimulation or the method of suspension.

At 4 days post slaughter, no differences in pH were seen ($P > 0.05$) while L^* value was again higher in the stimulated muscle relative to the non-stimulated ($P < 0.001$). Carbon dioxide stunning resulted in lower a^* ($P < 0.01$) and b^* ($P < 0.05$) values at this time but no differences ($P > 0.05$) in a^* or b^* were seen at rigor.

5.3.1.8 Cooking loss and purge

At both 4 and 10 days post slaughter, electrical stimulation and electrical stunning increased ($P < 0.01$) the level of purge from the muscle relative to non-stimulated and carbon dioxide stunned muscles respectively (Table 5.5). The method of suspension did not alter ($P > 0.05$) the purge levels at either 4 or 10 days post slaughter. Cooking loss at rigor, 1, 2, 8 and 10 days post slaughter was not influenced ($P > 0.05$) by method of stunning, method of suspension or the use of electrical stimulation. However, at 4 and 6 days post slaughter, cooking loss was influenced by carbon dioxide stunning with muscles from electrically stunned pigs having a lower cooking loss ($P < 0.05$). Also at 6 days post slaughter, the pelvic suspended muscles had a lower cooking loss ($P < 0.05$) than the Achilles suspended muscles not accounting for losses from purge.

5.3.2 Experiment 8: Influence of rate of pH and temperature decline on the rate of ageing of pork

5.3.2.1 Sample description

The average live weight of the pigs was 89.4 ± 22.67 kg. This resulted in an average carcass weight of 64.6 ± 18.14 kg and a fat depth at the P_2 site of 19.7 ± 6.9 mm. No sides could be classified as DFD while 6 sides could be classified as PSE ($n = 2$ – stimulated for 60 seconds, chilled at 2°C (33% of carcasses); $n = 2$ – stimulated for 60 seconds, chilled at 14°C (33% of carcasses); $n = 1$ – stimulated 15 seconds, chilled 2°C (16% of carcasses); $n = 1$ – stimulated 15 seconds, chilled at 14°C (16% of carcasses)) using the definition of PSE and DFD described in Warner *et al.* (1993).

5.3.2.2 pH and temperature decline

The rate of pH decline post slaughter was influenced ($P < 0.05$) by the application of low voltage electrical stimulation with carcasses stimulated for 60 sec having a faster rate of pH decline than all other treatments (Figure 5.5). No differences ($P > 0.05$) in the rate of pH decline were observed between those stimulated for 15 seconds and those not stimulated regardless of the method of stunning. Sides that received 60 sec electrical stimulation, carbon dioxide stunning and held at 14°C showed a trend ($P = 0.06$) towards a faster rate of pH decline relative to all other treatments.

Furthermore, muscles receiving 60 sec low voltage electrical stimulation, carbon dioxide stunning and held at 2°C had a faster rate of pH decline than those receiving 15 sec low voltage electrical stimulation or no stimulation regardless of stunning method or chilling temperature ($P < 0.05$).

At 30 minutes, and at each measurement time between 1 and 7 hours post slaughter, the pH was influenced ($P < 0.01$) by low voltage electrical stimulation. At 30 minutes post slaughter, stimulation for 15 seconds and 60 seconds reduced the pH relative to the non-stimulated carcasses ($P < 0.01$). By 1 hour post slaughter, the carcasses stimulated for 60 seconds had the lowest pH compared to those stimulated for 15 seconds and those not stimulated while those stimulated for 15 seconds had a lower pH than those not stimulated ($P < 0.01$). This same pattern was seen at 2, 3 and 6 hours post slaughter while at 4, 5 and 7 hours post slaughter there were no differences between 15 second and 60 second stimulation with both treatments having a lower pH than those not stimulated ($P < 0.01$). At 2, 3, 4, 5, 6 and 7 hours post slaughter the sides chilled at 14°C had a lower pH relative to the sides chilled at 2°C ($P < 0.01$ for all).

The time taken for the LTL muscle at all three sites to reach rigor was reduced by chilling at 14°C relative to chilling at 2°C ($P < 0.01$). Muscles which were stimulated for 60 seconds reached rigor pH faster than those stimulated for 15 seconds which in turn reached rigor pH faster than those which were not stimulated ($P < 0.001$) (Table 5.6).

The rate of temperature decline (k) was not influenced by any of the treatments ($P > 0.05$) (Figure 5.6).

At 30 minutes post slaughter, the sides that were stimulated for 15 seconds had a lower temperature than those which were not stimulated and stunned with carbon dioxide ($P < 0.05$). At 1 hour post slaughter, the sides chilled at 2°C had a lower ($P < 0.05$) temperature in the LTL than those chilled at 14°C and this pattern was also observed at 2, 3, 4, 5, 6 and 7 hours post slaughter ($P < 0.05$ for all).

5.3.2.3 Sarcoplasmic protein solubility, sarcomere length and Myofibrillar Fragmentation Index

At rigor, no differences in sarcoplasmic protein solubility were observed along with no differences in MFI (Table 5.6). At 4 days post slaughter muscles chilled at 14°C had a higher MFI than those chilled at 2°C ($P < 0.01$). The percentage change in MFI from rigor to 4 days post slaughter was increased by chilling at 14°C compared to chilling at 2°C ($P < 0.05$; Table 5.6).

At rigor, sarcomere length was shorter in muscles which had undergone carbon dioxide stunning and no stimulation relative to those electrically stunned with no stimulation and carbon dioxide stunned with electrical stimulation for 60 seconds ($P < 0.05$) (Table 5.6). Muscles from carcasses electrically stunned and held at 14°C also had longer sarcomere lengths relative to all samples stunned in carbon dioxide regardless of chilling temperature and those electrically stunned and chilled at 2°C ($P < 0.05$). By 4 days post slaughter, muscles that were chilled at 14°C had a longer sarcomere length relative to those chilled at 2°C ($P < 0.05$). Muscles taken from carcasses that were stunned in carbon dioxide and received no stimulation had shorter sarcomere lengths than all other treatments ($P < 0.01$).

At rigor there was no correlation between MFI and sarcomere length ($r = -0.199$, $P > 0.05$), MFI and WBSF ($r = -0.199$, $P > 0.05$) or WBSF and sarcomere length ($r = -0.216$, $P > 0.05$). At 4 days post slaughter there was a correlation between sarcomere length and WBSF ($r = -0.601$, $P < 0.05$) but no correlations between WBSF and MFI ($r = 0.069$, $P > 0.05$) or MFI and sarcomere length ($r = 0.010$, $P > 0.05$) were observed.

5.3.2.4 Protein Degradation:

A representative SDS-PAGE gel for the different treatments can be seen in Figure 5.7. A comparison of protein degradation in myofibril samples between rigor and 4 days post slaughter using SDS-PAGE illustrates that ageing occurred over this time period with changes in nebulin and titin bands. A faint nebulin band is still present at 4 days post slaughter in the muscles that had undergone carbon dioxide stunning and received no stimulation. A difference in the titin band in the rigor samples following chilling at 2°C can be seen with only a single band occurring in the myofibril

samples from muscles following no stimulation regardless of stunning method while a doublet appears in the samples following either long or short stimulation. In addition an extra band appears below myosin in the samples which received electrical stimulation and chilled at 2°C which could be a breakdown product of titin (Fritz and Greaser, 1991). This additional band also appears in the muscles chilled at 14°C although the double band for titin is not evident. The 4 day samples for the muscles chilled at 14°C following short or long periods of stimulation have a fainter titin band relative to the non-stimulated samples suggesting titin breakdown. It is not clear if a 30 kD band has appeared by 4 days post slaughter and whether troponin T has been degraded from rigor to 4 days post slaughter from these gels.

5.3.2.5 Warner-Bratzler shear force, ageing rate and sensory analysis

WBSF results are presented in Table 5.7. At rigor there were no differences between treatments for WBSF ($P > 0.05$). At 1 day post slaughter and all subsequent days all samples chilled at 14°C were more tender than those chilled at 2°C ($P < 0.001$). At 1 and 4 days post slaughter, the LTL of carcasses undergoing carbon dioxide stunning and 60 second electrical stimulation was more tender ($P < 0.01$) than the carbon dioxide stunned and no electrical stimulation treatments. At 4 days post slaughter, the LTL muscles that were stimulated for 15 seconds were also more tender than the muscles that were not stimulated and stunned with carbon dioxide ($P < 0.05$).

The rate of change in WBSF over an 8 day ageing period was not influenced by the method of stunning, the temperature of chilling or the use of low voltage electrical stimulation ($P > 0.05$) (Fig 5.8). The percentage change in WBSF from rigor to 4 days post slaughter was increased by chilling at 14°C compared to 2°C ($P < 0.05$) but was not influenced by stimulation ($P > 0.05$; Table 5.7).

At 2 days post slaughter, the sensory panel found the carcasses chilled at 14°C were more tender than those chilled at 2°C ($P < 0.01$) (Table 5.8). Also those stimulated for 60 seconds were more tender than those stunned with carbon dioxide and not stimulated ($P < 0.06$). No differences in flavour ($P > 0.05$) were detected but the electrically stunned, chilled at 14°C were juicier than the electrically stunned, chilled at 2°C and all carbon dioxide stunned samples regardless of the chilling temperature ($P < 0.05$).

The sensory panel found that samples chilled at 14°C were overall more acceptable than those chilled at 2°C ($P < 0.01$). The samples which were electrically stunned and chilled at 14°C were more acceptable to the panel than those electrically or carbon dioxide stunned and held at 2°C ($P < 0.05$).

A linear relationship was observed to occur between WBSF and taste panel tenderness responses ($\text{WBSF} = 0.06 \text{ tenderness} + 3.6$, $R^2 = 0.11$, $P < 0.05$), between WBSF and taste panel overall acceptability responses ($\text{WBSF} = 10.7 - 0.08 \text{ acceptability}$; $R^2 = 0.13$; $P < 0.01$) and between taste panel tenderness responses and overall acceptability ($\text{tenderness} = 112.2 - 1.1 \text{ acceptability}$; $R^2 = 0.80$; $P < 0.001$). Correlations between WBSF and tenderness response were significant ($r = 0.327$, $R^2 = 8.8$, $P < 0.05$). A linear relationship between sarcomere length measured at rigor and taste panel tenderness responses was observed. The regression equation is: $\text{sarcomere length} = 2.12 - 0.006 \text{ tender}$ ($P < 0.05$, $R^2 = 0.08$).

5.3.2.6 Colour, pH and drip loss

The surface colour, pH and drip loss determined at rigor pH and at 4 days post slaughter can be seen in Table 5.9. At rigor, carcasses from carbon dioxide stunned animals which did not receive electrical stimulation had a higher pH ($P < 0.05$) than those which were carbon dioxide stunned and stimulated for either 15 or 60 seconds. At rigor, muscles chilled at 14°C had a higher ($P < 0.05$) temperature than those chilled at 2°C. Carcasses receiving no low voltage electrical stimulation and electrically stunned had a lower temperature ($P < 0.05$) than muscles that were carbon dioxide stunned and stimulated for 60 seconds.

At rigor, muscles stimulated for 60 seconds and carbon dioxide stunned had a higher L^* value ($P < 0.05$) relative to muscles that received no stimulation and stunned either electrically or using carbon dioxide. Muscles that were carbon dioxide stunned and stimulated for 60 seconds had a higher a^* values ($P < 0.05$) than those carbon dioxide stunned and stimulated for 15 seconds and those stunned with carbon dioxide with no electrical stimulation. There were no differences ($P > 0.05$) in b^* values for the different treatments.

At 4 days post slaughter, samples which had been stunned in carbon dioxide and electrically stimulated had a lower pH ($P < 0.05$) than those which had not been stimulated regardless of the stunning method. No differences were seen in L^* values ($P > 0.05$) but samples from carcasses stimulated for 60 seconds had a redder appearance relative to those stimulated for 15 seconds or not stimulated at all as indicated by the a^* value ($P < 0.01$). Muscles chilled at 14°C resulted in a higher b^* value ($P < 0.05$) at 4 days post slaughter relative to those chilled at 2°C . Muscles from carcasses that had been stimulated for 60 seconds also had a higher b^* value ($P < 0.05$) than those stimulated for 15 seconds or did not receive low voltage electrical stimulation.

Drip loss at rigor was higher for muscles that underwent stimulation for 60 seconds than for samples which received no stimulation ($P < 0.05$). Muscles that had experienced electrical stunning with no stimulation had a lower drip loss than muscles from carcasses stimulated for both 15 and 60 second stimulated muscles ($P < 0.05$).

5.3.2.7 Purge and cooking loss

At 4 days post slaughter, purge levels were lower for the electrically stunned, no stimulation samples relative to those collected after carbon dioxide stunning and electrical stimulation for either 15 or 60 seconds ($P < 0.05$). Purge was also lower in those which had been stunned in carbon dioxide and received no stimulation relative to those which had been stimulated for 60 seconds ($P < 0.05$) (Table 5.10). Essentially the same pattern for the effect of treatment on purge was seen at 8 days post slaughter.

At rigor, cooking loss was lower in the carbon dioxide, no stimulation muscles relative to those electrically stunned and those stunned in carbon dioxide and stimulated for either 15 or 60 seconds ($P < 0.01$) (Table 5.11). At 1 and 2 days post slaughter, no differences were seen in cooking loss. By 4 days post slaughter, the carbon dioxide stunned, no stimulation treated muscles had a higher cooking loss than those that were stimulated for either 15 or 60 seconds ($P < 0.05$). By 8 days post slaughter, no differences in cooking loss were observed ($P > 0.05$).

5.3.3 Experiment 9: Influence of rate of pH decline on the rate of ageing of lamb

5.3.3.1 Sample description

The average live weight of the lambs slaughtered was 50.5 ± 3.67 kg. This resulted in an average carcass weight of 26.0 ± 2.07 kg with an average fat depth at the GR site of 18.4 ± 3.40 mm.

5.3.3.2 Rate of pH and temperature decline

The rate of pH decline (k) was not influenced by electrical stimulation or the method of suspension ($P > 0.05$ for both). The equations for each treatment can be seen in Figure 5.9 and the relationship between pH and time fits an exponential decay ($R^2 = 48.0$ to 98.4 , $RSD = 0.04$ to 0.195 , $P < 0.05$).

The average pH for the three sites measured at 40 minutes, and from 1 to 7 hours post slaughter was lower at each time point for electrically stimulated carcasses compared to non-stimulated carcasses ($P < 0.01$ for all times). The method of suspension did not influence ($P > 0.05$) the pH at any of these times.

The time taken to reach rigor was reduced by stimulation with the non-stimulated carcasses taking 10.5 hours to reach rigor while the stimulated carcasses required 7.2 hours ($P < 0.01$). The method of suspension had no influence ($P > 0.05$) on the time taken to reach rigor.

The rate of temperature decline was not influenced by electrical stimulation, or the method of suspension ($P > 0.05$). The average rate of temperature decline for all lambs can be seen in Figure 5.10. No differences in temperature at each time point were observed ($P > 0.05$).

5.3.3.3 Sarcomere length, myofibrillar fragmentation index

Sarcomere length measured at rigor was longer in the electrically stimulated muscles relative to the non-stimulated muscles ($P < 0.01$) (Table 5.11). However, sarcomere length was not influenced by the method of suspension ($P > 0.05$).

Electrical stimulation and the method of suspension did not influence the myofibrillar fragmentation index assessed at rigor (Table 5.11).

There were no correlations between WBSF and MFI ($r = 0.198$, $P > 0.05$), WBSF and sarcomere length ($r = -0.276$, $P > 0.05$) or MFI and sarcomere length ($r = 0.175$, $P > 0.05$).

5.3.3.4 Protein Degradation

A representative SDS-PAGE gel for the different treatments can be seen in Figure 5.11. A comparison of protein degradation in myofibril samples between rigor and 4 days post slaughter using SDS-PAGE illustrates that ageing occurred over this time period. However, no differences in the ageing patterns were observed between the treatments. Nebulin was degraded between rigor and 4 days post slaughter in all treatments but it is unclear if titin and troponin T have been degraded due to the clarity of the bands in the gel. A faint 30kD band also appears in the 4 day samples for all treatments.

5.3.3.5 Sarcoplasmic and myofibrillar protein solubility

Sarcoplasmic and myofibrillar protein solubility measured at 1, 3 and 5 hours post slaughter and rigor were not influenced by electrical stimulation or method of suspension ($P > 0.05$ for both) indicating that there were no differences in sarcoplasmic and myofibrillar protein denaturation (Table 5.12). A greater effect may have been seen if protein solubility was measured with KCl as described in method 1, protein solubility in Chapter 2.

5.3.3.6 Warner-Bratzler shear force and rate of ageing

The WBSF values measured over a 10 day ageing period can be seen in Table 5.13. WBSF at rigor, 1, 2, 4, 6, 8 and 10 days post slaughter were all lower with electrical stimulation relative to the muscles which were not stimulated ($P < 0.05$). At 1, 4 and 10 days post slaughter, WBSF was lower with pelvic suspension relative to Achilles suspension ($P < 0.05$) and a similar trend was seen at 6 and 8 days post slaughter ($P < 0.1$).

The rate of ageing over a 10 day ageing period was not influenced by stimulation ($P > 0.05$). Carcasses suspended by the pelvis tended to age at a faster rate than the Achilles suspended carcasses ($P < 0.1$) (Figure 5.12). The percentage change in WBSF from rigor to 4 days post slaughter was not influenced by either stimulation or suspension method ($P > 0.05$, Table 5.13).

5.3.3.7 Colour and pH

The effect of treatments on colour and pH at rigor and at 4 days post slaughter are given in Table 5.14. At rigor, there was no differences between treatments in either pH or L^* value ($P > 0.05$). The stimulated muscles had a higher a^* value ($P < 0.01$) and a higher b^* value ($P < 0.05$) than the non-stimulated muscles. The stimulated, Achilles suspended muscles were also redder than the 3 other treatments ($P < 0.01$) and had a higher b^* value relative to the non-stimulated, Achilles suspended muscles ($P < 0.01$).

At 4 days post slaughter, no differences ($P > 0.05$) in pH were seen between the different treatments while stimulated muscles had a higher L^* relative to the non-stimulated muscles ($P < 0.05$). The stimulated muscles maintained their higher a^* value relative to the non-stimulated muscles ($P < 0.001$) while those suspended by the pelvis had a higher a^* value than those suspended by the Achilles ($P < 0.05$). Again the stimulated muscles had a higher b^* value than the non-stimulated muscles ($P < 0.01$).

5.3.3.8 Cooking loss and purge

At 4 and 10 days post slaughter, electrical stimulation and the method of suspension (Table 5.15) did not influence the level of purge from the LTL muscle. Cooking loss at rigor and 1, 2, 4, 6 and 10 days post slaughter was not influenced by the use of electrical stimulation ($P > 0.05$). However, at 8 days post slaughter, cooking loss was reduced by electrical stimulation relative to those not stimulated ($P < 0.05$). At rigor, 1, 2, 6 and 8 days post slaughter, the method of suspension did not influence cooking loss but at 4 and 10 days post slaughter the Achilles suspended muscles had a higher cooking loss than the pelvic suspended muscles ($P < 0.05$).

5.3.4 Pigs from experiments 7 and 8 grouped according to rate of pH decline post slaughter

The results when grouped according to the rate of pH decline can be seen in Table 5.16.

The pH at 30 minutes, 1, 2 and 3 hours post slaughter were all influenced by the rate of pH decline ($P < 0.05$ for all) with the fast group having the lowest pH at each time with no differences in pH between the medium and slow groups. At 4, 5 and 6 hours post slaughter the fast group had the lowest pH and the pH of the medium group was lower than the slow group ($P < 0.05$ for all). The fast group reached rigor more quickly ($P < 0.001$) than all other groups. The rates of pH decline for the fast group was quicker than the rate of pH decline of the other two groups ($P < 0.001$) and the medium group had a quicker rate than the slow group ($P < 0.001$) (Figure 5.13).

The rate of ageing was not influenced by the rate of pH decline ($P > 0.05$; Figure 5.14).

At rigor, 1, 4 and 8 days post slaughter, WBSF was reduced in the fast group relative to the other two groups ($P < 0.05$) while at 2 days post slaughter the slow group had a higher WBSF than the fast group ($P < 0.01$).

At rigor, the pH of the fast group was lower than the other two groups ($P < 0.001$). At 4 days post slaughter, the fast group had a lower pH than the slow and medium groups ($P < 0.01$). The fast group also had a higher L^* value at rigor and 4 days post slaughter than the other groups ($P < 0.001$). Drip loss measured at rigor was higher in the fast groups relative to the other two groups ($P < 0.001$).

No differences were observed in sarcoplasmic protein solubility at rigor ($P > 0.05$). At rigor no differences were seen in MFI ($P > 0.05$) but at 4 days post slaughter the fast group had a lower MFI than the medium group ($P < 0.05$). At rigor and 4 days post slaughter the fast group had a longer sarcomere length relative to the slow and medium groups ($P < 0.01$ for all).

5.3.5 Lambs from experiment 9 grouped according to rate of pH decline

Very few differences between pH decline groups were seen in the measurements when the lambs were grouped in this fashion. The results can be seen in Table 5.17.

The WBSF values were only influenced by the rate of pH decline at 2, 8 and 10 days post slaughter with the medium pH decline group having the most tender lamb at this time period relative to the slow group ($P < 0.01$; Figure 5.16). No differences in overall rate of ageing were seen when the lambs were grouped according to the rate of pH decline ($P > 0.05$).

The pH from 1 to 7 hours post slaughter was reduced in the medium decline group relative to the slow group ($P < 0.05$; Figure 5.15) and so was the time taken to reach rigor ($P < 0.05$). The only other difference observed was a lower sarcoplasmic protein solubility in the medium pH decline group relative to the slow group at rigor ($P < 0.01$).

5.4 DISCUSSION

In previous experiments (experiments 2 and 5) differences in tenderness between rigor boned muscles occurred and it was postulated that this was caused by the rate of pH and temperature decline post slaughter as the breed, age, and pre-slaughter state of the animals was identical. The differences in tenderness between experiments was believed to be due to cold shortening conditions occurring in experiment 5 due to the pH being greater than 6 when muscle temperature fell below 10°C but not occurring in experiment 2. The discovery that muscle shortening is one of the major causes of meat toughness has lead to the realisation that post slaughter treatments will often outweigh factors such as breed, age, stress and pre-slaughter state in determining palatability (Polidori *et al.*, 1996). Thus experiments 7 and 8 were designed to examine the effect of the rate of pH and temperature decline on the rate of ageing of pork and experiment 9 was designed to determine if lamb muscle responded in a similar manner to that of pork.

The rate of pH and temperature decline can influence tenderness due to two main factors. If the rate of pH and temperature decline are such that the temperature falls below 10°C while muscle pH is still above 6.0, cold shortening will occur (Hamm,

1981). Furthermore, the cold conditions may also reduce proteolytic activity thus further reducing ageing. On the other hand, if the rate of pH decline is relatively fast with a slow temperature decline, protein denaturation will occur (Wismer-Pedersen, 1959). Due to changes in the protein structure that occur with denaturation, proteolysis is reduced and again ageing will be minimised. It is also possible that denaturation of the tenderising enzymes themselves occurs following a fast rate of pH decline which would also prevent ageing. Thus these experiments were designed to determine the influence of both cold shortening and protein denaturation on tenderness and subsequent ageing by inducing a fast and slow pH decline.

The pork and lamb data was grouped according to the rate of pH decline regardless of the treatment to determine the 'true' effect of rate of pH decline on the rate of ageing. When the pigs were grouped according to their rate of pH decline, the rate of ageing was not related to the rate of pH decline. However at each time measured the pork WBSF values were significantly related to the rate of pH decline. The fast rate of pH decline group generally had a lower WBSF than the slow and medium rate of pH decline groups.

The results found in experiments 7 and 8 support previous findings where the fast rate of pH decline ($\text{pH}_3 < 6.2$) in veal results in the most tender meat relative to the slow ($\text{pH}_3 > 6.7$) groups (Klont *et al.*, 1996). Although the pH at 3 hours post slaughter were much higher in the study by Klont *et al.* (1996) than those observed in experiments 7 and 8 the same trends were still observed. However, the results for WBSF when the animals are grouped according to their rate of pH decline are in contrast to that of Marsh *et al.* (1987) where the rate of ageing was reported to be greatest when intermediate rates (pH at 3 hours post slaughter = 6.1) of glycolysis occurred in veal with rapid (pH at 3 hours post slaughter = 5.38) and slow (pH at 3 hours post slaughter = 6.99) glycolytic rates corresponding to tougher muscles. The means by which the intermediate rate of pH decline was obtained did not seem to matter in the study by Marsh *et al.* (1987). However, the differences between the study by Marsh and the results presented here can be explained by the vast differences between the groups in the two studies. The corresponding pH at 3 hours post slaughter for the different pork groups for experiments 7 and 8 were slow = 6.15, medium = 6.08 and fast = 5.72 which are greatly different to those of Marsh *et al.* (1987). Furthermore, Pike *et al.* (1993) reported the same trend as Marsh *et al.*

(1987) for intermediate rates (pH = 6.0 at 3 hours post slaughter) of pH decline resulting in the most tender muscle.

Similar to the results for pork, when the lambs were grouped according to the rate of pH decline the rate of ageing was similar for both groups, again disproving the original hypothesis that the rate of pH decline will influence the rate of ageing. Due to the treatments for lamb not inducing a rapid rate of pH decline, only a slow and medium group could be assessed. Unlike that seen for pork, the rate of pH decline influenced WBSF values at 2, 8 and 10 days post slaughter with lower WBSF occurring in the medium rate of pH decline group while only at 8 days post slaughter was there a difference in WBSF between the slow and medium pork groups. Thus it can be concluded that the rate of pH decline in lamb has a greater influence on WBSF than the rate of pH decline in pork.

The rate of pH and temperature decline post slaughter can be influenced by many factors. These factors include breed (Judge *et al.*, 1989), pre-slaughter handling (D'Souza, 1998), stunning method (Barton-Gade, 1993), processing time (D'Souza, 1998), use of electrical stimulation (Kastner *et al.*, 1993; Takahashi *et al.*, 1984), and chilling conditions (Dransfield and Lockyer, 1985). In these experiments, different stunning methods, electrical stimulation and chilling temperatures were used to manipulate pH and temperature decline and ultimately determine their influence on meat tenderness and ageing. The outcomes are highlighted in the discussion presented below.

5.4.1 Experiment 7: Influence of rate of pH decline on the rate of ageing of pork

5.4.1.1 Rate of pH and temperature decline

Two methods were used to alter the rate of pH decline post slaughter in experiment 7, the first being the method of stunning and the second being the use of low voltage electrical stimulation. Carbon dioxide and electrical stunning using head to heart tongs were employed. Channon *et al.* (1997) and Barton-Gade (1993) reported that carbon dioxide stunning slowed down the rate of pH decline relative to electrical stunning. The differences in rate of pH decline due to stunning method have been

attributed to differences in the response of the pigs and their musculature to the method of stunning (Channon *et al.*, 1997; Barton-Gade, 1993). The faster rate of pH decline post slaughter of the electrically stunned pigs is believed to be induced by the application of the current itself and by the struggling which occurs while the animal is losing consciousness (McLoughlin, 1971). In contrast to what was expected, the method of stunning did not influence the rate constant for pH decline in this study but the muscle pH was lower for electrical stunning at some of the measurement times post slaughter than for carbon dioxide stunning. Although no differences in the rate constants were observed for pH decline, the electrically stunned pigs reached rigor 2 hours faster than those stunned in carbon dioxide suggesting the method of determining the rate of pH decline was not valid.

The second method employed to alter the rate of pH decline was the use of low voltage electrical stimulation. Electrical stimulation has been used extensively with beef and lamb carcasses to accelerate the rate of pH decline (Shaw *et al.*, 1996, Pike *et al.*, 1993, Taylor and Tantikov, 1992 and Devine *et al.*, 1984), prevent cold shortening and reduce the length of ageing required (Pike *et al.*, 1993; Shaw *et al.*, 1996). However electrical stimulation has not been used as extensively in pork due to the risk of inducing PSE conditions, increasing protein denaturation and thereby possibly preventing ageing. Similar to other reports of increased pH decline with electrical stimulation, low voltage electrical stimulation in experiment 7 resulted in a faster rate of pH decline. This faster rate of pH decline for the electrical stimulated sides resulted in a reduction in the time to reach rigor by 4 hours relative to the non-stimulated carcasses. Thus the use of electrical stimulation was an effective treatment to examine the effect of pH decline on the rate of ageing.

In this study, the rate of temperature decline was constant across all treatments. The 0-2°C chilling conditions used in Experiment 7 were sufficient to induce cold shortening in the non stimulated sides. This shortening usually occurs when a temperature of less than 10°C occurring in the muscles before the pH falls below 6.0. As the stimulated sides reached pH 5.8 prior to the muscle temperature falling below 10°C, shortening should have been prevented. Thus the chilling conditions were an effective treatment to examine the influence of cold shortening on the rate of ageing for the muscles which were not electrically stimulated.

5.4.1.2 Protein denaturation, proteolysis and cold shortening

The rate of pH decline is believed to influence tenderness when either protein denaturation occurs due to a fast pH fall, the proteolytic rate is altered (Dransfield, 1994; Geesink, 1993; Ouali and Talmont, 1990; Etherington, 1989) or cold shortening occurs due to slow pH fall (Davey *et al.*, 1969). These factors can be assessed by protein solubility; SDS-PAGE and MFI; and sarcomere length respectively. Thus it was hypothesised that the use of electrical stunning and electrical stimulation would induce a rapid pH fall and protein denaturation while the use of carbon dioxide stunning without electrical stimulation would induce cold shortening. However, the use of electrical stunning did not alter the rate of pH decline post slaughter therefore little impact of stunning was observed. No differences in sarcomere length, myofibrillar fragmentation, protein denaturation or protein degradation due to stunning method were induced. This disproves the original hypothesis that electrical stunning might increase sarcomere length, MFI and protein denaturation due to an expected higher rate of pH decline relative to carbon dioxide stunning. Only at rigor was myofibrillar protein solubility higher in muscles from electrically stunned pigs relative to those stunned in carbon dioxide. The use of preslaughter stress or halothane sensitive pigs may have been a more effective method of inducing protein denaturation than electrical stunning and low voltage stimulation.

Although electrical stimulation did alter the rate of pH decline, there were no differences in myofibrillar and sarcoplasmic protein solubility indicating the rate of pH decline was not fast enough to induce PSE or the temperature was not high enough when the ultimate pH was reached to induce PSE. The lack of protein denaturation following electrical stimulation is probably due to the efficient chilling employed in this experiment, reducing carcass temperatures quickly thereby preventing the lower pH conditions from causing denaturation of the proteins. Although, electrical stimulation has been reported to increase protein denaturation, the increase in this denaturation has been small and difficult to measure (Pearson and Dutson, 1985). Thus the impact of protein denaturation on the rate of ageing could not be determined and the hypothesis that protein denaturation prevents ageing could not be proven. Although little denaturation was detected, 6 muscles could be classified as PSE, mainly when electrical stunning and electrical stimulation were employed indicating that some protein denaturation may have occurred due to these treatments but not to the extent of being detectable in the protein solubility results. However 2 muscles which were

not stimulated also showed PSE characteristics which may also help to explain why differences in denaturation were not detected.

Unlike the results seen for protein denaturation, differences in sarcomere length were observed between the different treatments indicating the rate of pH decline may influence sarcomere length. Due to the chilling conditions employed, cold shortening was induced as indicated by a reduction in sarcomere length, in those samples that did not receive electrical stimulation. The increased rate of pH decline induced by electrical stimulation overcame the cold shortening that occurred in the muscles which did not receive stimulation as indicated by longer sarcomeres.

Cold shortening in slow pH decline muscles is thought to be avoided by stretching the muscles by altering the method of carcass suspension for example via pelvic suspension (tenderstretching). Pelvic suspension resulted in increased sarcomere length relative to Achilles suspension, indicating the prevention of cold shortening. The increase in sarcomere length due to electrical stimulation was equivalent to the increase in sarcomere length following pelvic suspension thus both electrical stimulation and pelvic suspension are effective methods of preventing cold shortening. This increased sarcomere length and improved tenderness due to pelvic suspension in experiment 7 has also been reported by Hostetler *et al.* (1975) in beef, and by Moller *et al.* (1987) and Moller and Vestergaard (1986) in pork. Thus pelvic suspension can be considered as an alternate method to electrical stimulation to prevent cold shortening in pork loin muscle but the impact on the leg muscles needs to be investigated.

Finally, the impact of the rate of pH decline on proteolytic activity was not what would be expected with no differences occurring between the treatments in proteolytic degradation as assessed by MFI and only slight differences occurring in the protein degradation patterns seen on the SDS-PAGE. Only slight differences in nebulin degradation were observed with slower degradation occurring in the muscles that received carbon dioxide stunning and no electrical stimulation relative to the other treatments. The faster rate of pH decline with electrical stimulation was expected to result in increased MFI and protein degradation due to the activation of the proteolytic enzymes but this did not appear to be the case. Thus the hypothesis that improvements in tenderness due to increased proteolytic activity in the intermediate

pH group is disproven. The lack of differences seen in degradation patterns of titin and troponin T is consistent to the results of Ho *et al.* (1997), who also observed no increase in protein degradation of titin, nebulin or desmin following electrical stimulation in beef. Thus from these results it is clear that the main influence that electrical stimulation had on pork muscle was the prevention of severe cold shortening as minimal differences were observed in protein degradation patterns and MFI indicating that electrical stimulation did not influence proteolytic activity. The lack of extensive differences in proteolytic degradation patterns as assessed by SDS-PAGE may be due to the lower sensitivity of this method of assessment and the fast rate of ageing seen in pork. The majority of ageing in pork has occurred by 4 days post slaughter (Dransfield *et al.*, 1980-81), thus differences may not be detectable by SDS-PAGE at this late stage of the ageing process. Therefore, differences may have been detectable if samples had been collected at earlier times post slaughter.

The impact of the change in pH decline as a result of electrical stimulation on cold shortening is further evident when the muscles are grouped according to the rates of pH decline. The muscles that fell into the slow rate of pH decline had undergone considerable cold shortening as indicated by shorter sarcomere length. Although the impact of cold shortening on tenderness could be assessed when the muscles were grouped according to the rate of pH decline, the impact of protein denaturation could not as the rate of pH decline was not sufficient to induce protein denaturation although some PSE was detected in both stimulated and non-stimulated muscles.

5.4.1.3 Warner-Bratzler peak shear force and rate of ageing

It was originally hypothesised that WBSF and the rate of ageing would be influenced by the rate of pH decline due to a slow rate of pH decline inducing cold shortening and a fast rate of pH decline inducing protein denaturation. The impact of increased pH decline rate induced by electrical stimulation on tenderness was indicated by the rate of ageing, the percentage change in WBSF and the WBSF values themselves. The rate of ageing and the percentage change in WBSF was increased by low voltage electrical stimulation. Furthermore, WBSF values at 2 days post slaughter in the electrically stimulated carcasses only occurred at equivalent values in the non-stimulated carcasses by 10 days post slaughter. The improvements in tenderness post slaughter with electrical stimulation are believed to be due to the faster rate of

pH decline preventing cold shortening as indicated by the longer sarcomere lengths following electrical stimulation. However electrical stimulation did not appear to promote proteolytic activity as indicated by SDS-PAGE and MFI as originally hypothesised indicating that electrical stimulation does not promote proteolytic activity as would be expected due to lower muscle pH occurring at higher muscle temperature. This may be due to the chilling temperature being sufficient to cause shortening of the muscles when no stimulation is employed but not sufficient to prevent proteolytic activity.

The improvements in tenderness due to increased pH decline is consistent with studies on the effect of glycolytic rate in beef by Pike *et al.* (1993) and Savell (1979) who reported that electrically stimulated carcasses required less ageing than non-stimulated carcasses to reach the same level of tenderness. Pike *et al.* (1993) showed that the rate of glycolysis was the primary determinant of LTL tenderness and that temperature decline rate was only important through its influence on early post slaughter glycolytic rate. Furthermore, the rate of ageing is increased following electrical stimulation due to the proteolytic enzymes acting much faster as a consequence of the accompanying structural changes following stimulation (Pearson and Dutson, 1985). However, there was no change in rate of proteolysis following electrical stimulation in experiment 7 with no differences in MFI occurring between the treatments and only minor differences in nebulin degradation being observed in the protein degradation patterns.

The improvements in tenderness following electrical stimulation have been previously reported for pork with most of these studies involving rapid chilling (-20 to -40°C for several hours post slaughter followed by chilling at 2°C). Taylor and Tantikov (1992) and Taylor *et al.* (1995) have shown that electrical stimulation with rapid chilling results in improved meat tenderness, PSE prevention and carcass weight loss reduction. However, the improvements in pork tenderness due to electrical stimulation seen after chilling at 0-2°C in experiment 7 are in contrast to other studies using slower chilling temperatures where improvements in tenderness were not observed as both slow chilling and electrical stimulation will result in increased proteolytic activity early post slaughter due to a low pH while muscle temperature is still high. A study by Warriss *et al.* (1995) in pigs using slow chilling methods (ambient temperature for 2 hours then into 2°C) reported that many of the benefits to

tenderness of electrical stimulation with rapid chilling were lost under these conditions. Takahashi *et al.* (1984) reported that rapid acidification of beef due to electrical stimulation resulted in increased toughness after slow chilling (37°C until 3 hours post slaughter then chilled at 2°C). This toughening was believed to be due to the impact of temperature in promoting proteolytic activity being less when the tissue is nearing its ultimate pH than when high temperature and high pH occur together. Thus it is believed by Takahashi *et al.* (1984) that electrical stimulation is only effective in improving tenderness when chilling conditions are likely to cause cold shortening or high voltage electrical stimulation causes fibre rupture as fast pH decline on its own is actually detrimental to the tenderising process. However, the contrast in improvements in tenderness following electrical stimulation and slow chilling between Takahashi *et al.* (1984) studies in beef and the improvements in pork tenderness at chilling temperatures of 0-2°C in experiment 7 may be explained by the difference in glycolytic and temperature decline rates between beef and pork and cold shortening conditions occurring at this chilling temperature in the pork muscles. Due to the faster rate of pH fall post slaughter in pork and the occurrence of cold shortening in this experiment, the effect of temperature on proteolytic activity may not be as pronounced in pork in this experiment. Furthermore, the conditions employed by Warriss *et al.* (1995) and Takahashi *et al.* (1984) may have been sufficient to induce protein denaturation thereby reducing proteolytic activity.

The different stunning methods used induced an interesting response in the rate of ageing in experiment 7. The electrically stunned muscles produced a typical shaped ageing curve as was seen in experiment 2 and previously by Dransfield *et al.* (1980-81). In contrast, the shape of the ageing curve was altered by carbon dioxide stunning. The variation in shape due to carbon dioxide stunning may be due to high pH following carbon dioxide stunning early post slaughter causing differences in the initial tenderness values at 1 and 2 days post slaughter. In addition, the electrically stunned muscles come close to final WBSF by 4 to 5 days relative to 10 days post slaughter following carbon dioxide stunning. The longer time required for the carbon dioxide muscles to reach rigor relative to electrically stunned muscles would probably explain the trend towards shorter sarcomere length. This trend towards muscle shortening was sufficient to reduce the ageing rate for carbon dioxide stunned muscles as indicated by WBSF and reduced proteolytic activity as indicated by less nebulin degradation. Temperature conditioning at 14°C following carbon dioxide

stunning in experiment 8 seemed to be an effective method of producing a traditional ageing pattern. The effect of chilling at 14°C on the shape of the ageing curve may be due to the reduction in time taken to reach rigor relative to chilling at 2°C thereby reducing the incidence of cold shortening and the impact that cold shortening has on the rate of ageing.

Pelvic suspension prevented cold shortening as seen in the general reduction in WBSF at various times post slaughter and the increase in sarcomere length in experiment 7. Although improvements in WBSF were observed with pelvic suspension, the rate of ageing was not altered by the method of suspension. At 10 days post slaughter similar WBSF values had been reached by pelvic (4.6 kg) suspended and by stimulated (4.5 kg) muscles in experiment 7. This finding is similar to that of Taylor *et al.* (1992a) who reported that high voltage electrical stimulation and pelvic suspension were equally effective in improving pork tenderness. However in experiment 7, 10 days of ageing was still required with pelvic suspension to produce a level of tenderness acceptable to the consumer (WBSF < 5.0 kg, Shorthose *et al.*, 1986).

5.4.1.4 Surface colour and water holding capacity

In addition to influencing tenderness, the rate of pH decline can also influence water holding capacity and colour. Both a fast and slow rate of pH decline can increase drip loss due to the likely occurrence of protein denaturation following a fast rate of pH decline and the possibility of cold shortening with a slow rate of pH decline. Furthermore, protein denaturation as a result of a fast rate of pH decline is likely to induce a higher L* value while the cold shortening as a result of a slow pH decline may induce a lower L* value. In this experiment, colour and some components of water holding capacity were detrimentally affected by electrical stimulation.

Electrical stimulation resulted in faster pH decline and an increase in purge, without any effect on drip loss. The increased purge level following electrical stimulation can probably be attributed to increased myofibrillar weakening with post slaughter storage relative to non stimulated muscles as indicated by the higher nebulin degradation thereby increasing purge but not drip loss. Due to increased fragmentation of the myofibrils with post slaughter storage, free water is lost from the muscles thus

increasing purge levels of the stimulated muscles. Taylor and Tantikov (1992) also saw the lack of influence on drip loss in pork loin by electrical stimulation. As PSE and protein denaturation were not induced by the faster rate of pH decline, it is not surprising that drip loss was similar for the electrically stimulated and non-stimulated muscles. However, drip loss values for both stimulated and non-stimulated muscles were high relative to drip loss values in experiments 4 and 5 (2.21% and 1.25% respectively). The lack of difference in drip loss due to electrical stimulation can be explained by the short stimulation period employed and the efficient chilling process preventing protein denaturation, thereby preventing an increase in drip loss. Furthermore the lack of difference in drip loss between stimulated and non-stimulated muscles may be due to the cold shortening that occurred in the non-stimulated muscles. In experiments 3 and 4 drip loss was increased by cold shortening as the increased muscle contraction resulted in squeezing of the water out of the muscle. Thus the cold shortening observed in the non-stimulated muscles may have increased the drip loss as suggested by higher drip loss values (3.04%) relative to those observed previously in non-shortened muscle (2.21% for AB-14 muscles in experiment 4, 1.25% for AB-14 muscles in experiment 5).

Conflicting reports in the literature exist regarding the influence of electrical stimulation on drip loss. The differences in drip loss reports may be explained by the variation in pH and temperature decline resulting from different forms of stimulation, time of stimulation post slaughter, the length of time applied and the chilling conditions employed. Chilling at 1°C was reported by Taylor *et al.* (1992a) to increase drip loss relative to initial chilling at -20°C following electrical stimulation. Increased drip loss was again seen by Taylor *et al.* (1992b) when low voltage stimulation was applied immediately post slaughter for 90 seconds relative to muscles which were not electrically stimulated regardless of the chilling method (1°C or rapid chilling). However in the same study, delaying stimulation (high or low voltage) to 20 minutes post slaughter followed by rapid chilling resulted in the same level of drip loss as the non-stimulated muscles (Taylor *et al.*, 1992b). Increased drip loss in pork after electrical stimulation has also been reported by Warriss *et al.* (1995), Taylor and Martoccia (1995), and Dransfield *et al.* (1991). Thus the level of drip loss following electrical stimulation is strongly influenced by the type of stimulation (high or low voltage, the time of application post slaughter and the length

of time applied) along with the chilling conditions employed. Therefore it is difficult to compare the impact of electrical stimulation on drip loss across studies.

Muscles from animals that were electrically stunned had an increased drip loss relative to the animals that were carbon dioxide stunned. This increased drip loss due to electrical stunning was not associated with an increased protein denaturation, a difference in rate of pH decline or a reduction in sarcomere length. Although the rate of pH decline was not altered by the method of stunning, electrical stunning reduced the time taken to reach rigor and also the muscle pH at 1, 2 and 3 hours post slaughter. Thus the reduction in absolute pH early post slaughter may be playing a greater influence on muscle structure than the overall rate of pH decline as the pH/temperature history will be shifted towards more denaturation due to the lower initial temperature, thereby increasing drip loss. Furthermore, a higher number of electrically stunned carcasses could be classified as PSE relative to carbon dioxide stunned carcasses. This increased drip loss may be due to increased muscular contraction observed in the electrically stunned carcasses immediately following stunning thereby damaging muscle structure and resulting in a higher drip loss (Barton-Gade *et al.*, 1990).

The L^* value was increased by low voltage electrical stimulation probably as a result of the lower pH with electrical stimulation early post slaughter. The increased L^* value due to electrical stimulation is a consistent finding with that seen in beef (Aalhus *et al.*, 1994). This increased L^* value is most likely due to the acceleration of post slaughter metabolism combined with the lower pH observed at rigor. Lower muscle pH is believed to increase free water at the cell surface resulting in an increased reflectance giving the meat a lighter appearance (Pearson and Dutson, 1985). Furthermore, the higher L^* value following electrical stimulation could be due to a greater amount of light being reflected from the muscle as a result of a looser structure occurring, also allowing deeper oxygen penetration (Unruh *et al.*, 1986). However, the increased L^* value following electrical stimulation was not a result of protein denaturation.

Thus from experiment 7 it can be seen that the use of low voltage electrical stimulation was an effective method of increasing the rate of pH decline. By inducing a faster rate of pH decline, cold shortening was prevented and tenderness was

improved. However, the faster rate of pH decline did not alter the rate of proteolytic activity as indicated by myofibrillar fragmentation index and the few differences seen in the protein degradation patterns on SDS-PAGE with only minor differences in nebulin degradation being observed. Furthermore, the faster rate of pH decline did not induce PSE conditions or protein denaturation as measured by protein solubility, thus the impact of protein denaturation on pork tenderness could not be assessed. Furthermore, the faster rate of pH decline induced by electrical stimulation did not increase drip loss but it did increase purge and surface lightness.

Carbon dioxide stunning did not alter the rate of pH decline relative to electrical stunning but the time taken to reach rigor was longer with carbon dioxide stunning. Electrical stunning resulted in a trend towards longer sarcomere lengths and a reduction in WBSF at 1 and 2 days post slaughter relative to muscles from carcasses that received carbon dioxide stunning. Although time to reach rigor was reduced following electrical stunning, again protein denaturation was not induced and proteolytic activity was not altered but drip loss was increased.

In conclusion, experiment 7 did not support the hypothesis originally proposed whereby a fast rate of pH decline will prevent ageing due to protein denaturation and an intermediate rate of pH decline will improve the rate of ageing due to the prevention of cold shortening and the increased activation of the proteolytic enzymes. However, the hypothesis that a slow rate of pH decline will result in cold shortening and meat which is tougher and takes longer to age was proven.

5.4.2 Experiment 8: Influence of rate of pH and temperature decline on the rate of ageing of pork

Due to the absence of protein denaturation in experiment 7, the treatments imposed in experiment 8 were designed to induce the extremes of protein denaturation and cold shortening. It was hypothesised that by inducing a rapid rate of pH decline with a slow rate of temperature decline, protein denaturation would occur, thus allowing the influence of protein denaturation on the rate of ageing to be determined and compared to the slower rate of pH decline that induces cold shortening. To induce the faster rate of pH decline, a longer electrical stimulation period than what was

used in experiment 7 was applied to the carcass and a less rapid chilling rate was also employed.

5.4.2.1 Rate of pH and temperature decline

Low voltage electrical stimulation resulted in the faster rate of pH decline as originally postulated with the sides stimulated for 60 seconds having a faster pH decline rate than those stimulated for 15 seconds or not stimulated at all. However, no differences were observed between the non-stimulated sides and those stimulated for 15 seconds in the pH rate constants which contrasts with the results seen in experiment 7. This difference between experiments 7 and 8 may be because the pigs in experiment 8 which received low voltage electrical stimulation were all carbon dioxide stunned whereas those in experiment 7 were electrically stunned and stimulated. Channon *et al.* (1997) previously reported that electrically stunned pigs had a lower muscle pH at 1 hour post slaughter relative to carbon dioxide stunned pigs. Although the rate constant for the pH decline was not increased by stimulation for 15 seconds relative to no stimulation, these sides still had a lower pH (approximately 0.2 pH units) than the non-stimulated muscles at each time measured.

The temperature of chilling played an important role in the fall in pH post slaughter. Lower muscle pH values along with higher temperatures occurred in the muscles chilled at 14°C relative to the muscles chilled at 2°C. Due to the slower decline in muscle temperature as a result of the higher chiller temperature, the enzymatic activity of the glycolysing enzymes would have been promoted due to the increase in ATP hydrolysis as a result of higher temperatures thereby resulting in a faster fall in pH (Pike *et al.*, 1993). This increased glycolytic enzyme activity would have caused the observed reduction in time taken to reach rigor. Pike *et al.* (1993) also reported that temperature was important in influencing pH decline in beef. Pike *et al.* (1993) postulated that subcutaneous fat depth may play an important role in temperature decline post slaughter and found that leaner bulls had a lower temperature at 3 hours post slaughter relative to fatter bulls. The higher pH can be attributed to the reduction in glycolytic activity due to lower muscle temperature. Thus from both experiment 8 and the study by Pike *et al.* (1993) it can be seen that muscle temperature plays an important role in determining glycolytic rate.

Cold shortening has been reported to occur if muscle temperature falls below 10°C while the pH is above 6.0 (Dransfield, 1994). The 0-2°C chilling conditions used in experiment 8 induced cold shortening in the non stimulated sides as indicated by a reduction in sarcomere length. The stimulated sides reached pH 6.0 prior to the muscle temperature falling below 10°C which prevented cold shortening as evident in the longer sarcomere length relative to those that were not stimulated. Furthermore, all muscles chilled at 14°C also had not undergone cold shortening as indicated by the longer sarcomere lengths.

5.4.2.2 Protein denaturation, proteolysis and cold shortening

Experiment 8 was designed to determine if protein denaturation had any effect on the rate of ageing. It was postulated that by applying stimulation for a longer period and chilling at a higher temperature, differences in protein denaturation would result. Protein denaturation is believed to occur when a rapid rate of pH decline occurs while muscle temperature is still high (Wisner-Pederson, 1959; Greaser *et al.*, 1969; Fischer *et al.*, 1979). Therefore, by inducing a fast rate of pH decline by stimulating the carcasses for 60 seconds along with inducing a slower rate of temperature decline by chilling at 14°C, extensive protein denaturation was expected. However, no differences in sarcoplasmic protein solubility were seen in experiment 8 as had previously been the case in experiment 7. This lack of difference may be due to the muscles pH decline not being fast enough to induce PSE conditions and protein denaturation. Extreme PSE and protein denaturation is reported to occur when a pH of 5.8 occurs at 45 minutes post slaughter (Warner, 1994), conditions which were not observed in either experiment 7 or 8. Thus the influence of protein denaturation on the rate of ageing could not be investigated. In retrospect, preslaughter stress or the use of halothane sensitive pigs may have been a better method of inducing faster pH decline rates relative to electrical stimulation due to their known influences on accelerating glycolysis, inducing PSE and protein denaturation (D'Souza, 1998; Channon *et al.*, 1997).

Again, the impact of the rate of pH decline on cold shortening and protein degradation were examined to determine the impact on the rate of ageing. Similar results for sarcomere length measurements to those observed in experiment 7 were

again observed here. Cold shortening occurred as a result of the slower rate of pH decline induced in the muscles that were not stimulated as indicated by shorter sarcomere length and higher WBSF at 1 and 4 days post slaughter. No differences in MFI at rigor were observed indicating no early differences in protein degradation. However, differences in the level of proteolytic activity as determined by the protein degradation patterns on SDS-PAGE were observed with a greater level of titin degradation following electrical stimulation.

At 4 days post slaughter, MFI and the percentage change in MFI were higher as a result of chilling at 14°C indicating protein breakdown increased for muscles chilled at 14°C. This confirms that the muscle temperature rather than the rate of pH decline may be important in influencing the level of proteolytic activity as originally hypothesised as both glycolytic and proteolytic activity are temperature dependent. The higher temperatures of the muscles chilled at 14°C would have enabled the activity of the proteolytic enzymes to increase as reported by Dransfield (1994), Geesink (1993) and Ouali and Talmont (1990). Furthermore, the higher temperature prevented shortening as indicated by the longer sarcomere length thereby the proteolytic enzymes had a greater capability of degrading the proteins (Iversen *et al.*, 1995). Dransfield *et al.* (1992a) indicated that tenderisation starts when muscles reach a pH of approximately 6.1. As the muscles chilled at 14°C reached rigor faster than those chilled at 2°C, the proteolytic enzymes would have been activated earlier thereby increasing myofibrillar fragmentation. Again, the lack of extensive differences in proteolytic degradation patterns between chilling temperatures as assessed by SDS-PAGE as seen in experiment 7 may be due to the lower sensitivity of this method of assessment and the fast rate of ageing seen in pork. Therefore, differences may have been detectable if samples had been collected at earlier times post slaughter. From these results it appears that chilling at 14°C promotes proteolytic activity possibly due to both the increased muscle temperature early post slaughter relative to chilling at 2°C and the lower pH observed in the muscles from 2 hours post slaughter. Thus supporting the original hypothesis that proteolytic activity will be increased when cold shortening conditions are prevented.

5.4.2.3 Warner-Bratzler peak shear force, taste panel assessment and rate of ageing

In contrast to the results of experiment 7, the rate of ageing in experiment 8 was not influenced by stimulation or chilling. However, from 1 day post slaughter to 8 days post slaughter, absolute WBSF values were reduced by temperature conditioning at 14°C relative to chilling at 2°C. Furthermore, the percentage change in WBSF between rigor and 4 days post slaughter was also increased with chilling at 14°C. This difference in tenderness due to chilling method was also observed by the taste panel. The improvements in tenderness with temperature conditioned muscles can be attributed to the prevention of cold shortening as indicated by longer sarcomeres at 4 days post slaughter relative to the 2°C chilled muscles along with increased proteolysis as indicated by an increase in MFI. Studies by Hostetler *et al.* (1975) in beef using 16°C as a conditioning temperature for 20 hours also found that shear force at 7 days post slaughter was reduced relative to 2°C although no differences in sarcomere length or ease of fragmentation were observed. These results again support the hypothesis that WBSF will be reduced due to increased proteolytic activity when cold shortening are prevented.

The effect of electrical stimulation on tenderness is highly dependent on the subsequent cooling rate (Marsh *et al.*, 1987). Marsh *et al.* (1987) reported that a very slow chilling rate (temperature at 3 hours post slaughter between 32.7 and 36.7°C) accelerated the already high rate of pH fall (pH at 3 hours post slaughter less than 6.0) and this was accelerated to such an extent to result in toughening relative to a moderate increase in glycolytic rate and the increased toughening persisted through 14 days of ageing. Although, Pike *et al.* (1993) found that the temperature decline of beef played an important role in determining the rate of pH decline, the temperature at 3 hours post slaughter had practically no relationship to WBSF. In contrast they found that the pH at 3 hours post slaughter had a quadratic relationship with WBSF with muscle tenderness maximised at pH_{3hrs} of 6.2.

The difference in response to chilling temperature between the studies by Takahashi *et al.* (1984), Marsh *et al.* (1987) and Pike *et al.* (1993) and in experiment 8 may be due to species differences. The three previous studies were conducted in beef muscle that contains a higher proportion of red muscle fibres than pigs. The LTL muscle of the pig has a higher content of fast twitch glycolytic fibres causing a faster

rate of pH decline, contain different isoforms of the contractile proteins and different isoforms of the proteinases (Pearson and Young, 1989). Thus the predominance of white fibres in the LTL pig muscle relative to the predominance of red fibres of the beef muscle may cause the difference in response to chilling conditions between species.

Furthermore, the results in experiment 8 for the effect of chilling temperature on tenderness are in contrast to previous work performed with pork using slow chilling. Slow chilling by holding pig sides at 10°C until 3 hours post slaughter then chilling at 1°C, resulted in no differences in tenderness when compared to sides that were chilled immediately at 1°C (Taylor *et al.*, 1992c). The differences between Taylor's study and experiment 8 can be explained by the 10°C chilling temperature in the study by Taylor *et al.* (1992c) not being high enough to alter the rate of pH decline. In contrast, chilling at 14°C reduced the time required to reach rigor in experiment 8. The pH at 45 minutes and 3 hours post slaughter were the same for the delayed chilling and immediately chilled treatments in the study by Taylor *et al.* (1992c) whereas experiment 8 showed differences in pH from 2 hours post slaughter.

Electrically stimulated beef carcasses have previously been reported to require less ageing than unstimulated carcasses in order to reach the same level of tenderness (Savell, 1979). This was observed to occur in this experiment with the tenderness at 8 days post slaughter in the non-stimulated carcasses being equivalent to that measured at rigor for the samples stimulated for 60 seconds and at 4 days post slaughter for the samples stimulated for 15 seconds.

The rate of pH decline has previously been reported to influence WBSF in beef and pork. In beef, Geesink *et al.* (1995) found that the rates of pH and temperature decline seem to be the main determinants of WBSF at 1 day post slaughter and fitted linear regression equations. Geesink *et al.* (1995) reported that as the pH at 45 minutes increased, beef tenderness decreased at 1 day post slaughter. Furthermore, Geesink *et al.* (1995) found that as the temperature at 45 minutes post slaughter increased, WBSF also increased at 1 day post slaughter. The increase in toughness with higher pH and temperature reflect toughening by protein denaturation at low pH and high temperature. At the other extreme, Dransfield *et al.* (1991) found that rapid chilling of the LTL to 10°C within 3 hours post slaughter resulted in increased

toughness of pork. Although chilling was important in influencing tenderness, Dransfield *et al.* (1991) found toughness was influenced more by carcass suspension and high voltage electrical stimulation than by rapid chilling. According to Dransfield (1992a) and Dransfield *et al.* (1992a, and b) tenderisation starts when muscles reach a pH of approximately 6.1, thus the slower glycolysing muscles start tenderising later than the fast glycolysing muscles leading to tougher muscles at 1 day post slaughter. Furthermore, proteolysis begins at higher muscle temperature in muscles with a faster glycolytic rate, thereby proteolysis will proceed faster

The tenderness results observed using WBSF were confirmed by the taste panel assessment at 2 days post slaughter. Both WBSF and sensory results showed that pork tenderness after 2 days ageing was improved by previous chilling of the carcass at 14°C relative to 2°C. Thus both sensory and WBSF results support the hypothesis that improvements in tenderness occur with a rate of pH decline which prevents cold shortening. There was a linear relationship between WBSF and taste panel tenderness response with only 11% of the variation in WBSF explained by taste panel tenderness response. WBSF values were also correlated to taste panel responses for overall acceptability. The linear response between WBSF and taste panel tenderness response results are consistent with those observed by Poste *et al.* (1993) and Bouton *et al.* (1975). Furthermore, the correlation between taste panel tenderness responses and overall acceptability suggests that consumers value tenderness as an important meat quality characteristic.

Electrical stimulation did not appear to influence the flavour or juiciness of the pork muscle as assessed by taste panel responses in experiment 8 which agrees with results of Bowles-Axe *et al.* (1983), Taylor (1979), Gilbert *et al.* (1977) and Gilbert and Davey (1976). However, the absence of an effect of electrical stimulation on flavour is in contrast to Savell (1979) who reported beef flavour to improve by 10% as a result of electrical stimulation. The flavour and juiciness results in experiment 8 are also in contrast to those of Warriss *et al.* (1995) who reported electrical stimulation followed by slow chilling decreased juiciness and increased pork flavour while no differences in tenderness were found along with no differences in overall eating quality. The reduced juiciness with electrical stimulation was attributed by Warriss *et al.* (1995) to an increased drip loss and the improved flavour with electrical

stimulation was speculated to be due to the increased formation of nucleotide flavour precursors due to rapid initial post mortem metabolism.

A linear relationship between sarcomere length and taste panel responses for tenderness were observed in experiment 8. This relationship indicated that as sarcomere length increased, tenderness also improved however, only 8% of the observed variation was accounted for by this equation. A linear relationship between taste panel tenderness response and sarcomere length for beef was observed by Smulders *et al.* (1990) but they reported the relationship accounted for 30% of the observed variability. Smulders *et al.* (1990) reported that as the sarcomere length decreased, the relationship deteriorated and eventually became valueless as a predictor of tenderness while long sarcomere lengths were a good predictor of tenderness. Furthermore, Smulders *et al.* (1990) found that in slow glycolysing muscles tenderness was highly dependent on shortening. However in muscles of more rapid pH decline tenderness was completely independent of shortening. Tenderness was optimised when glycolysis proceeded at an intermediate rate.

5.4.2.4 Surface colour and Water Holding Capacity

Drip loss was observed to increase with stimulation for 60 seconds in experiment 8 relative to non-stimulated muscles. The increased drip loss can be explained by the faster rate of pH decline due to the longer stimulation period as again no differences in drip loss between the non-stimulated, carbon dioxide stunned muscles and those stimulated for only 15 seconds was observed. This increased drip loss due to electrical stimulation is consistent to that previously reported by Dransfield *et al.* (1991), Taylor *et al.* (1992a, b) Warriss *et al.* (1995), and Taylor and Martoccia (1995) who also used longer stimulation periods than 15 seconds.

The increased surface L* value due to low voltage electrical stimulation seen at rigor in pork LTL is consistent with that seen in beef by Aalhus *et al.* (1994) and in experiment 7 and can be explained as described previously for experiment 7.

Low voltage electrical stimulation has also been associated with changes in a* and b* values (Warriss *et al.*, 1995). The increased a* value of the 60 second stimulation muscles in experiment 8 at rigor and 4 days post slaughter and b* at 4 days post

slaughter is consistent to that seen by Warriss *et al.* (1995) who also reported an increase in a^* and b^* values and a similar trend was observed in experiment 7 although a shorter stimulation period was employed.

Although, electrical stimulation influenced the meat colour, the temperature the carcasses were held at did not influence colour at rigor or at 4 days post slaughter. This is in contrast to the results of experiment 4 where temperature conditioning at 14 and 21°C resulted in a higher L^* and b^* value relative to 0 or 7°C following accelerated processing and results of Ledward (1985). Ledward (1985) reported that the temperature at which muscle goes into rigor and the time it is held at that temperature can modify the subsequent rate of metmyoglobin formation due to alterations in enzyme activity following cutting with a high metmyoglobin content resulting in a dull brown meat colour (Tinbergen, 1975).

Thus from this experiment it was again observed that the rate of pH decline was increased by electrical stimulation which resulted in reduced cold shortening and thereby improved tenderness again supporting the hypothesis that cold shortening needs to be prevented to improve pork tenderness. The temperature of chilling did not influence the rate of pH decline but the time to reach rigor was reduced by 1.3 hours with chilling at 14°C along with lower muscle pH values from 2 hours post slaughter. The reduced time to reach rigor for 14°C carcasses relative to 2°C may have contributed to the greater percentage change in WBSF from rigor to 4 days post slaughter and more tender pork from 2 days post slaughter due to the prevention of cold shortening and increased proteolytic activity. The higher chilling temperature increased proteolysis as indicated by the higher percentage change in MFI from rigor to 4 days post slaughter and the increased MFI at 4 days post slaughter relative to those chilled at 2°C. This supports the hypothesis that accelerating proteolytic activity will result in improved tenderness. However, once again protein denaturation was not induced so the impact of denaturation on tenderness could not be examined and the hypothesis that protein denaturation reduces ageing could not be addressed.

Furthermore, electrical stimulation for 60 seconds resulted in increased drip loss and a higher L^* value although no difference in sarcoplasmic protein denaturation was observed. This suggests that mild denaturation may have occurred due to the accelerated rate of pH decline following stimulation for 60 seconds stimulation but at

a level that was not detectable by sarcoplasmic protein solubility or some unknown mechanism has resulted in the increased drip loss. Furthermore, the level of denaturation did not appear to be sufficient to influence tenderness as was proposed in the original hypothesis.

5.4.3 Experiment 9: Influence of rate of pH decline on the rate of ageing of lamb

In the final experiment, the rate of pH decline in lamb was examined to determine if the rate of pH decline altered the rate of ageing of lamb in the same manner as that seen in pork. Due to the reported greater susceptibility of lamb to cold shortening than pork, the importance of the rate of pH decline in influencing the rate of ageing could be of greater significance in lamb than in pork. Furthermore, differences in the rate of proteolysis between lamb and pork have been reported due to differences in the fat thickness, rate of pH and temperature decline, initial tenderness and differences in the ratio of calpastatin to *m*-calpain (Koohmaraie *et al.*, 1991, Ouali and Talmont, 1990; Etherington *et al.*, 1987). Thus these differences along with the differences in fibre type between the two species may affect the ageing response to the rate of pH decline in lamb compared to pork.

5.4.3.1 Rate of pH and temperature decline

In contrast to the results for pork, low voltage electrical stimulation did not increase the rate constant for pH decline in the lamb carcasses which is in contrast to results obtained by Chrystall and Devine (1992, 1985). Although the rate of pH decline was not reduced, the pH at 40 minutes through to 7 hours post slaughter was reduced by stimulation and the time required to reach rigor pH was reduced by 3 hours with stimulation. This result parallels that seen for the 15 second stimulation for pork in experiment 8 while Hertog-Meischke *et al.* (1987) reported that lamb following low voltage electrical stimulation had a lower pH at 45 minutes post slaughter but not at 3 hours post slaughter. The rate constant for pH decline in the lamb muscle may not have been altered by electrical stimulation compared to pork due to the differences in muscle fibre type between the species. Pork muscle has a higher amount of white fast twitch glycolytic fibres compared to lamb muscles which have more red, slow twitch fibres (Pearson and Young, 1989). Thus, the pork muscles are more

susceptible to a faster rate of pH decline and therefore may potentially have a greater response to electrical stimulation (Devine *et al.*, 1984).

In experiment 9, the rate of temperature decline was constant across the treatments. Similar to that seen for pork, the 0-2°C chilling conditions were sufficient to induce cold shortening in the non-stimulated sides as muscle temperature fell below 10°C prior to a pH less than 6.0 occurring.

5.4.3.2 Protein denaturation, proteolysis and cold shortening

The same response to electrical stimulation was seen for sarcomere length, MFI, protein solubility and protein degradation as assessed by SDS-PAGE in lamb as previously seen in pork in experiments 7 and 8. As was reported earlier in pork, chilling at 0-2°C of the muscles that were not stimulated resulted in cold shortening as indicated by a reduction in sarcomere length and an increase in WBSF. However, pelvic suspension did not increase sarcomere length as was expected. This difference is probably due to the small carcass size of the lamb carcasses and lower subcutaneous fat levels increasing the rate of temperature decline relative to pork, which would increase the level of shortening. Thus the use of electrical stimulation is of greater importance in lamb to prevent cold shortening than for pork.

As the rate of pH decline for lamb was not increased by the use of electrical stimulation and due to the efficient chilling conditions used, no differences in protein denaturation were observed. Again these results were observed for pork even when longer stimulation was employed and chilling temperatures were increased to 14°C. Thus the impact of protein denaturation on tenderness could not be assessed. Similar results for lamb LTL were also observed by Hertog-Meischke *et al.* (1997) following low voltage stimulation (85V, 14Hz for 15 seconds immediately after slaughter) and chilling at 2.5°C overnight who found no differences in myofibrillar protein solubility between stimulated and non-stimulated muscles. However, in their case, a decrease in sarcoplasmic protein solubility did occur, indicating some denaturation of the sarcoplasmic proteins.

The influence of electrical stimulation on protein denaturation in experiment 9 is in contrast to those reported by Hector *et al.* (1992). Hector *et al.* (1992) reported an

increased level of myosin denaturation in lamb *semimembranosus* following low voltage electrical stimulation (90V for 30 seconds) and slow chilling which allowed the muscle temperature to fall to less than 7°C over 24 hours. The differences in protein denaturation between Hector *et al.* (1992) and experiment 9 may be explained by the slower chilling conditions used by Hector *et al.* (1992) and the different muscles used for assessment. The *semimembranosus* used in the study by Hector *et al.* (1992) would have experienced a slower muscle temperature decline than the LTL used in Experiment 9 thus increasing the chance of denaturation occurring. Furthermore, the response to electrical stimulation of the LTL muscle would be less than that of the *semimembranosus* due to the greater amount of fast twitch oxidative-glycolytic and fast twitch glycolytic fibres present in the *semimembranosus* which are more responsive to electrical stimulation (Pearson and Young, 1989).

MFI and protein degradation as assessed by SDS-PAGE did not differ between treatments as previously seen for pork in experiment 7. The lack of difference in proteolysis may be attributed to the fast chilling conditions that occurred regardless of the stimulation treatment thereby slowing down proteolysis. Therefore the main impact of electrical stimulation for lamb, like that for pork, was the prevention of cold shortening.

5.4.3.3 Warner-Bratzler peak shear force and rate of ageing

Similar results were seen for the ageing rate of lambs as was seen in experiment 8 for 15 and 60 second electrical stimulation for pork. Low voltage electrical stimulation did not influence the rate of ageing nor the percentage change in WBSF between rigor and 4 days post slaughter although stimulation did reduce the WBSF values at each time measured. It is probable that the lack of difference in the rate of ageing between electrically stimulated and non-stimulated muscles can be attributed to the lack of effect of electrical stimulation on the rate of pH decline. The influence of the effect of rate of pH decline on lamb tenderness is highlighted in the results when the lambs are grouped according to the rate of pH decline. At 2, 8 and 10 days post slaughter the medium rate of pH decline reduced WBSF which contrasts to that of pork where WBSF for the medium group was not different to that of the slow fast rate of pH decline group.

Unlike that seen in experiment 7 for pork, pelvic suspension of lamb showed a tendency towards increasing the rate of ageing and an increase in the percentage change in WBSF between rigor and 4 days post slaughter. Furthermore, pelvic suspension decreased WBSF at most time points of measurement, a similar result to that seen in pork in experiment 7 where WBSF was generally lower with pelvic suspension. The impact of carcass suspension on lamb tenderness is difficult to explain as the method of suspension did not alter the level of protein degradation, myofibrillar fragmentation or sarcomere length. As pelvic suspension has previously been reported to prevent muscle shortening (Taylor *et al.*, 1995; Moller and Vestergaard, 1986; Hostetler *et al.*, 1975) an increase in sarcomere length due to pelvic suspension was expected.

Electrical stimulation of lamb was more effective in improving tenderness than electrical stimulation of pork. The reduction in pork WBSF following stimulation was 1 to 1.6 kg at 1, 2 and 10 days post slaughter in experiment 7 and 2 to 2.4 kg at 1 and 4 days post slaughter in experiment 8. However, electrical stimulation of lamb carcasses resulted in 1.5 to 3 kg reduction in WBSF at all times measured post slaughter. This suggests that the lamb muscles response to electrical stimulation was greater than that for pork although the rate of pH decline was increased with stimulation in pork but not lamb. The differences in response between pork and lamb may be due to the faster rate of pH decline in pork relative to lamb when stimulation is not applied (Koochmarie *et al.*, 1991). Thus the impact of electrical stimulation may have been greater in lamb than pork due to the earlier activation of the tenderising enzymes accompanying the earlier onset of rigor. Furthermore, electrical stimulation usually has more effect in muscles with more red fibre types (Pearson and Young, 1989). As lamb muscles have a higher percentage of red fibres than pork the response to electrical stimulation may be greater (Pearson and Young, 1989).

The improvements in lamb tenderness with electrical stimulation is in contrast to that seen by both Unruh *et al.* (1986) and Takahashi *et al.* (1984). They both reported increased toughness after low voltage electrical stimulation. The difference in response to electrical stimulation may be explained by the difference in stimulation conditions used (Takahashi *et al.*, 1984 – 2 Hz, 500V at 40 minutes post slaughter and Unruh *et al.*, 1986 – 50V, 60 Hz for 2 minutes at 5 minutes post slaughter) and

the slower initial chilling employed by Takahashi *et al.* (1984) (37°C for 3 hours then chilled at 2°C).

5.4.3.4 Surface colour and water holding capacity

Finally, electrical stimulation altered lamb muscle colour but not water holding capacity as assessed by cooking loss and purge. An increased a^* value was observed in the electrically stimulated carcasses along with a higher b^* value. However, no differences were observed in L^* value. The colour results for lamb are in contrast to those seen in pork in experiment 7 but similar to experiment 8 with 60 seconds of electrical stimulation. The similar results of experiments 8 and 9 can be explained by similar length of time of application of electrical stimulation (60 and 50 sec respectively) relative to the shorter duration of stimulation in experiment 7 (15 sec). The difference in muscle fibre type between the species did not appear to alter the response in colour to stimulation with the magnitude of the changes similar for both species.

The lamb muscle colour response to electrical stimulation is in contrast to that seen by Unruh *et al.* (1986). Unruh *et al.* (1986) reported that beef that had received low voltage electrical stimulation was lighter in colour than control steaks as well as more yellow at 0 days post slaughter and less red at 5 days post slaughter than control steaks. These colour changes observed by Unruh *et al.* (1986) are a result of the rapid pH decline with electrical stimulation while temperature was still high. Unruh *et al.* (1986) reported more light was reflected from the surface following stimulation as a result of looser structure causing more light scattering and deeper oxygen penetration thus resulting in a lighter colour. Unruh *et al.* (1986) reported a reduction in water holding capacity and increased cooking loss as a result of low voltage electrical stimulation while in experiment 9 electrical stimulation did not increase cooking loss or purge.

From this experiment, it can be concluded that although the fibre types in lamb muscle differs to that of pork, the response to electrical stimulation was similar for both species. However, the rate of pH decline was not increased in lamb following electrical stimulation unlike that seen for pork nor was the rate of ageing increased following stimulation. Electrical stimulation of lamb prevented cold shortening but did

not induce protein denaturation or increased proteolytic activity. Furthermore, pelvic suspension resulted in improvements in tenderness but did not increase sarcomere length. Thus from this experiment it can be concluded that electrical stimulation is effective at preventing cold shortening in lamb thereby improving WBSF at each time measured.

5.5 CONCLUSIONS

The three experiments presented in this chapter were designed to determine the impact of the rate of pH and temperature decline on the rate of ageing of both pork and lamb. To assess this, the rate of pH decline was manipulated by the use of different stunning methods and the use of low voltage electrical stimulation. The rate of temperature decline was manipulated by the use of different chilling temperatures. By manipulating these conditions, the impact of cold shortening, altered protein degradation and protein denaturation on muscle tenderness, ageing and meat quality traits was determined.

Electrical stimulation applied to the carcass was an effective method to reduce the time taken to reach rigor in both pork and lamb. This reduction in time taken to reach rigor resulted in an increased rate of ageing in experiment 7 but not in experiments 8 and 9. Although the rate of ageing was not increased by electrical stimulation in experiments 8 and 9, improvements in WBSF did occur following stimulation. These improvements could be attributed to the prevention of cold shortening. Although electrical stimulation prevented cold shortening, it did not appear to increase protein degradation nor did it induce protein denaturation.

The use of different stunning methods did not influence the rate constant for pH decline but electrical stunning did reduce the time taken to reach rigor relative to carbon dioxide stunning. The reduction in time to reach rigor due to electrical stunning resulted in a reduction in WBSF at 1 and 2 days post slaughter. This improvement in WBSF was probably due to earlier activation of proteolytic enzymes as sarcomere length and protein denaturation were not influenced by the stunning method.

Although the method of stunning did not alter the rate of ageing, very different shaped ageing curves were obtained for the electrical stunned and carbon dioxide stunned animals that were chilled at 2°C. These differences can only be attributed to the reduction in time to reach rigor following electrical stunning as no differences in sarcomere length, protein denaturation or myofibrillar fragmentation were observed. The difference in ageing patterns between the stunning methods was not present for carcasses undergoing slower chilling at 14°C.

Differences in the rate of temperature decline appeared to have a greater impact on pork muscle tenderness than differences in pH decline. Chilling at 14°C reduced the time to reach rigor, reduced WBSF at all times measured and increased the percentage change in WBSF from rigor to 4 days post slaughter relative to chilling at 2°C. Again these improvements appear to be due to the prevention of cold shortening and increased myofibrillar fragmentation.

Finally, pelvic suspension was an effective method of overcoming the impact of cold shortening. By stretching the muscle, the reduction in sarcomere length was prevented, resulting in improvements in WBSF. Greater improvements occurred in sheep relative to pork which can be attributed to the greater susceptibility of lamb to cold shortening than pork due to the smaller carcass size and greater red fibre content of the muscle.

It was postulated that the intermediate rates of pH decline would produce the most tender meat due to the prevention of cold toughening and protein denaturation. However, when the animals were grouped according to their rate constants for pH decline, lower WBSF at 2, 8 and 10 days post slaughter were observed in the medium group relative to the slow group for lamb while the fast pH decline group for pork had the most tender meat at all times measured. These results reflect the fact that protein denaturation was not induced by any of the treatments as indicated by the protein solubility results thus the fast rate of pH decline was not fast enough to produce tougher muscle due to protein denaturation. However, the fast rate of pH decline was sufficient to induce PSE, thus indicating some protein denaturation, suggesting that the level of protein denaturation was not sufficient to influence tenderness.

A WBSF value of 5 kg has previously been suggested as a cut off point for consumer acceptability (Shorthose *et al.*, 1986). Using this cut off point and based on an internal cooking temperature of 80°C being obtained by the consumer, muscles that were electrically stunned and aged for 6 days in experiment 7 would be considered acceptable in tenderness to a consumer while ageing for 10 days is required following carbon dioxide stunning. Four days ageing was required for muscles following electrical stimulation in experiment 7 to reach an acceptable WBSF value and 6 days ageing was required when suspended by the pelvis while non-stimulated muscles and muscles removed from Achilles suspended sides did not obtain a WBSF less than 5 kg. When pork carcasses were grouped according to the rate of pH decline the fast group reached an acceptable tenderness within 4 days post slaughter while the other two groups failed to produce a consumer acceptable tenderness level over 10 days of ageing.

Very different results for consumer acceptability were observed in experiments 8 and 9 compared to experiment 7. No pork or lamb loins would be acceptable to the consumer even after ageing for 8 and 10 days respectively. When the lambs were grouped according to the rate of pH decline, an acceptable tenderness level was not produced in any group.

Finally the impact of the different treatments on meat quality was minimal with the most notable effect being the increased drip loss as a result of electrical stunning. The increased drip loss was attributed to possible membrane damage and the increased muscular contraction observed in the electrically stunned carcasses immediately following stunning relative to those stunned in carbon dioxide. Thus damage to the muscle structure may have occurred, thereby increasing drip loss. However, drip loss following electrical stimulation was only increased when 60 seconds of stimulation was applied.

Thus from these experiments, it can be concluded that low voltage electrical stimulation and higher chilling temperatures can be employed to improve meat tenderness due to reducing the time it takes for muscles to reach rigor. These improvements can be attributed to the prevention of cold shortening and under the conditions of these experiments protein denaturation was not a concern.

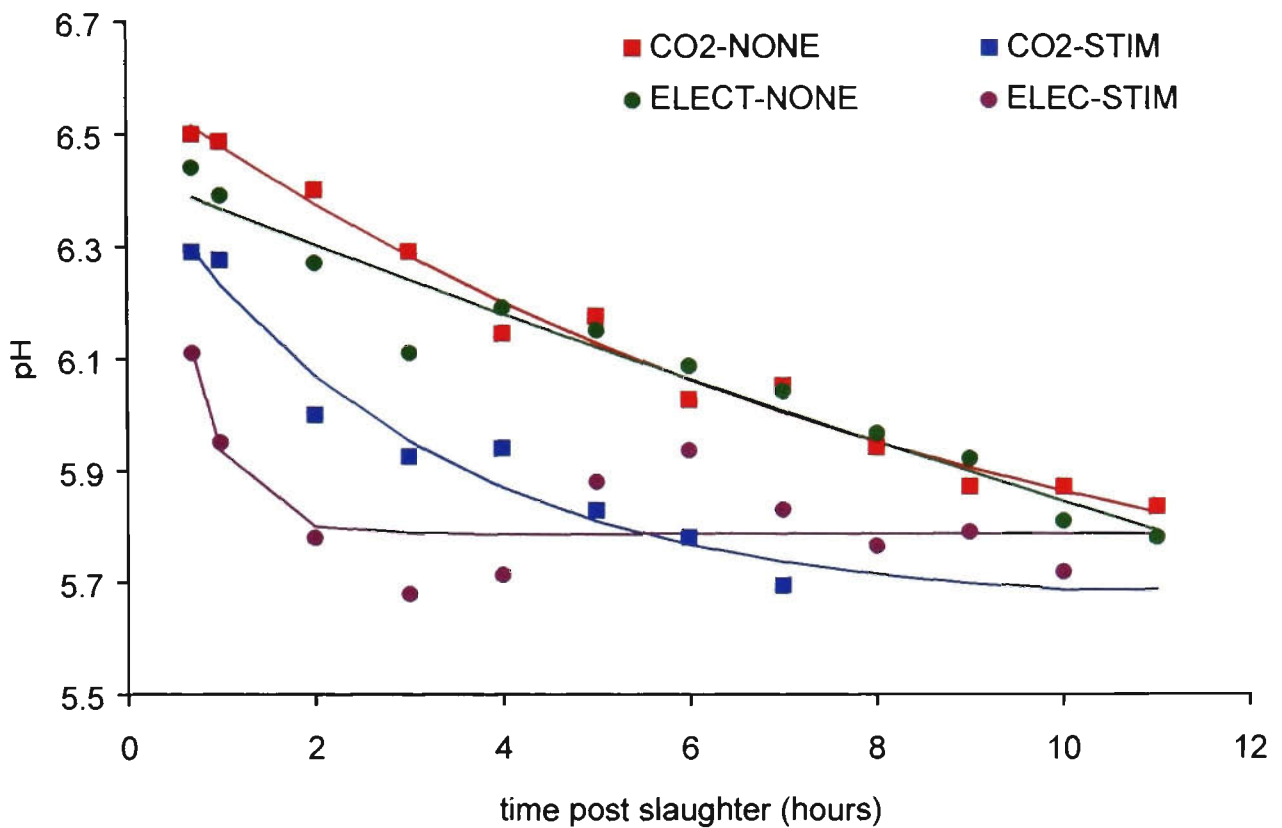


Figure 5.1 Mean rate of pH decline in experiment 7 for pork *M. longissimus thoracis et lumborum* after carbon dioxide (CO₂) or electrical head to heart stunning (ELECT), low voltage electrical stimulation (none (NONE) or for 15 seconds 5 minutes post slaughter (STIM)). Each point represents the actual pH at each time measured and the line represents the fitted curve.

The equations for each line are:

$$\text{pH}_{\text{CO}_2\text{-NONE}} = 5.517 + 1.078e^{-0.114t} (R^2=97.8, \text{RSD}=0.035),$$

$$\text{pH}_{\text{CO}_2\text{-STIM}} = 5.66 + 0.797 e^{-0.333t} (R^2=93.7, \text{RSD}=0.054),$$

$$\text{pH}_{\text{ELECT-NONE}} = 3.78 + 2.65 e^{-0.025t} (R^2= 93.4, \text{RSD}=0.054) \text{ and}$$

$$\text{pH}_{\text{ELECT-STIM}} = 5.79 + 1.64 e^{-2.41t} (R^2= 57.7, \text{RSD}=0.083).$$

The average sed = 0.108.

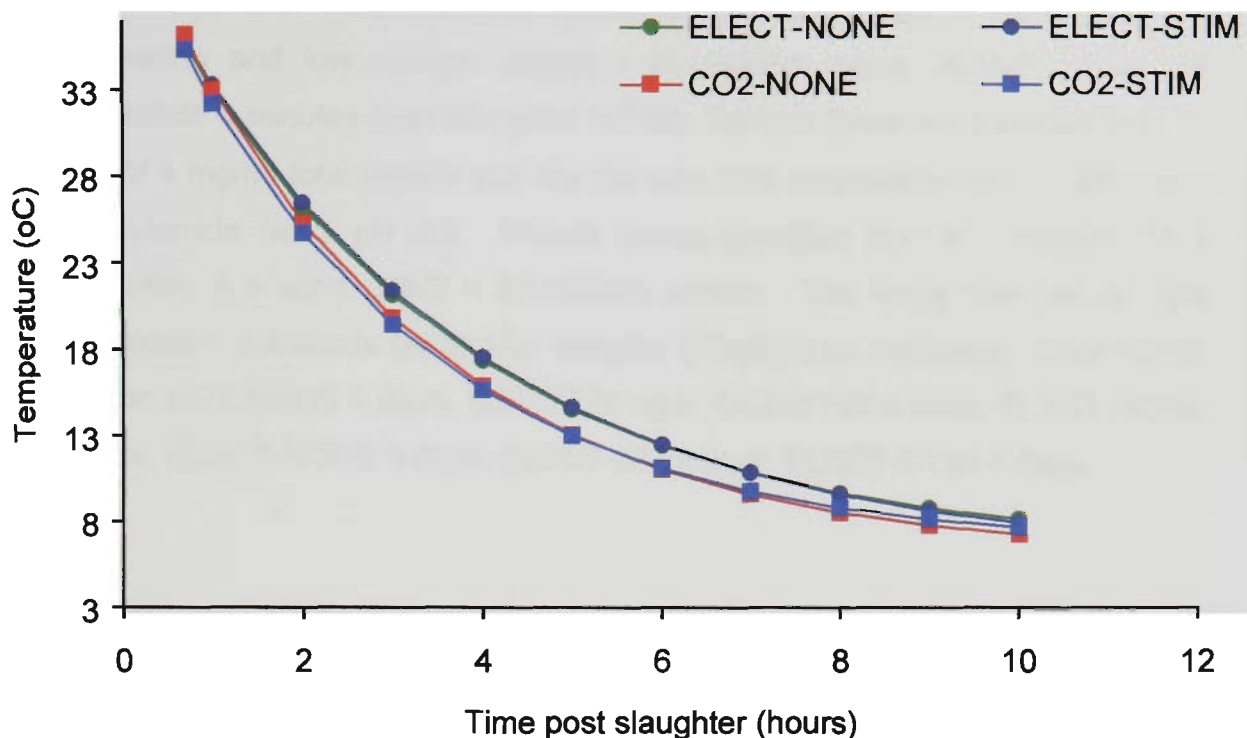


Figure 5.2 Mean rate of temperature (temp) decline in experiment 7 for pork *M. longissimus thoracis et lumborum* after carbon dioxide (CO₂) or electrical head to heart stunning (ELECT), low voltage electrical stimulation (none (NONE) or for 15 seconds 5 minutes post slaughter (STIM)). Each point represents the actual temperature at each time measured and the line represents the fitted curve.

The equations for each line are:

$$\text{temp}_{\text{CO2-NONE}} = 5.96 + 37.8 e^{-0.33t} (R^2=99.8, \text{RSD}=0.44),$$

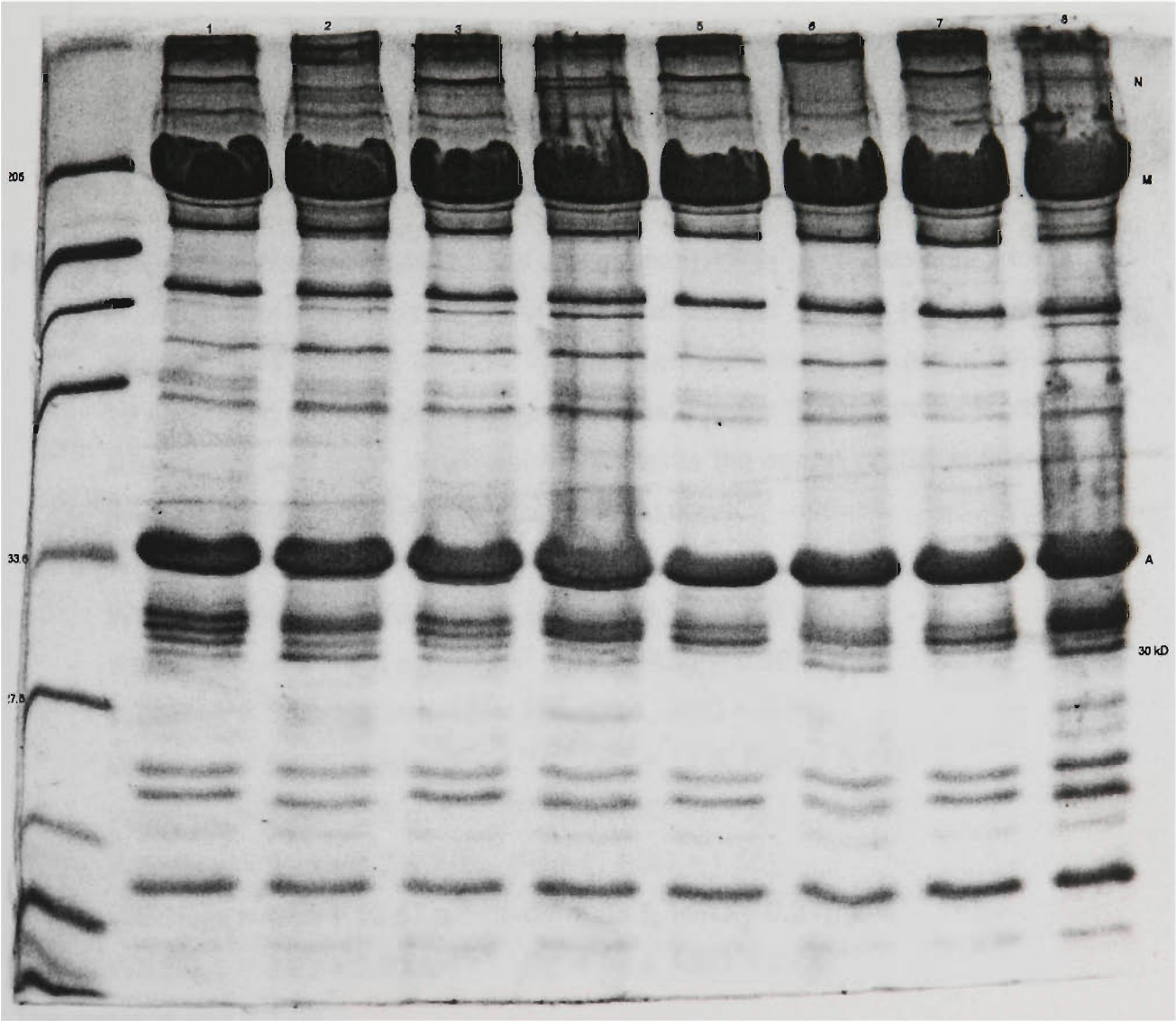
$$\text{temp}_{\text{CO2-STIM}} = 6.53 + 36.29 e^{-0.34t} (R^2=99.8, \text{RSD}=0.49),$$

$$\text{temp}_{\text{ELECT-NONE}} = 5.80 + 37.50 e^{-0.31t} (R^2= 99.9, \text{RSD}=0.37) \text{ and}$$

$$\text{temp}_{\text{ELECT-STIM}} = 5.80 + 36.47 e^{-0.28t} (R^2= 99.7, \text{RSD}=0.56).$$

The average sed = 1.20.

Figure 5.3 Representative SDS-PAGE showing the difference in protein bands following ageing in experiment 7 for pork *M. longissimus thoracis et lumborum* myofibrils after carbon dioxide (CO₂) or electrical head to heart (ELECT) stunning and low voltage electrical stimulation (none (NONE) or for 15 seconds 5 minutes post slaughter (STIM)). Sample lanes were loaded with 10 µl of 4 mg/ml total protein and the gel was 10% acrylamide (w/v), 0.06% bis-acrylamide (w/v), pH 9.3. Protein bands identified are; N = nebulin, M = myosin, A = actin, 30kD = 30 Kdalton protein. The lanes from left to right represent standards (molecular weights (kDalton) as indicated), CO₂-NONE rigor, CO₂-NONE 4 days, CO₂-STIM rigor, CO₂-STIM 4 days, ELECT-NONE rigor, ELECT-NONE 4 days, ELECT-STIM rigor, ELECT-STIM 4 days.



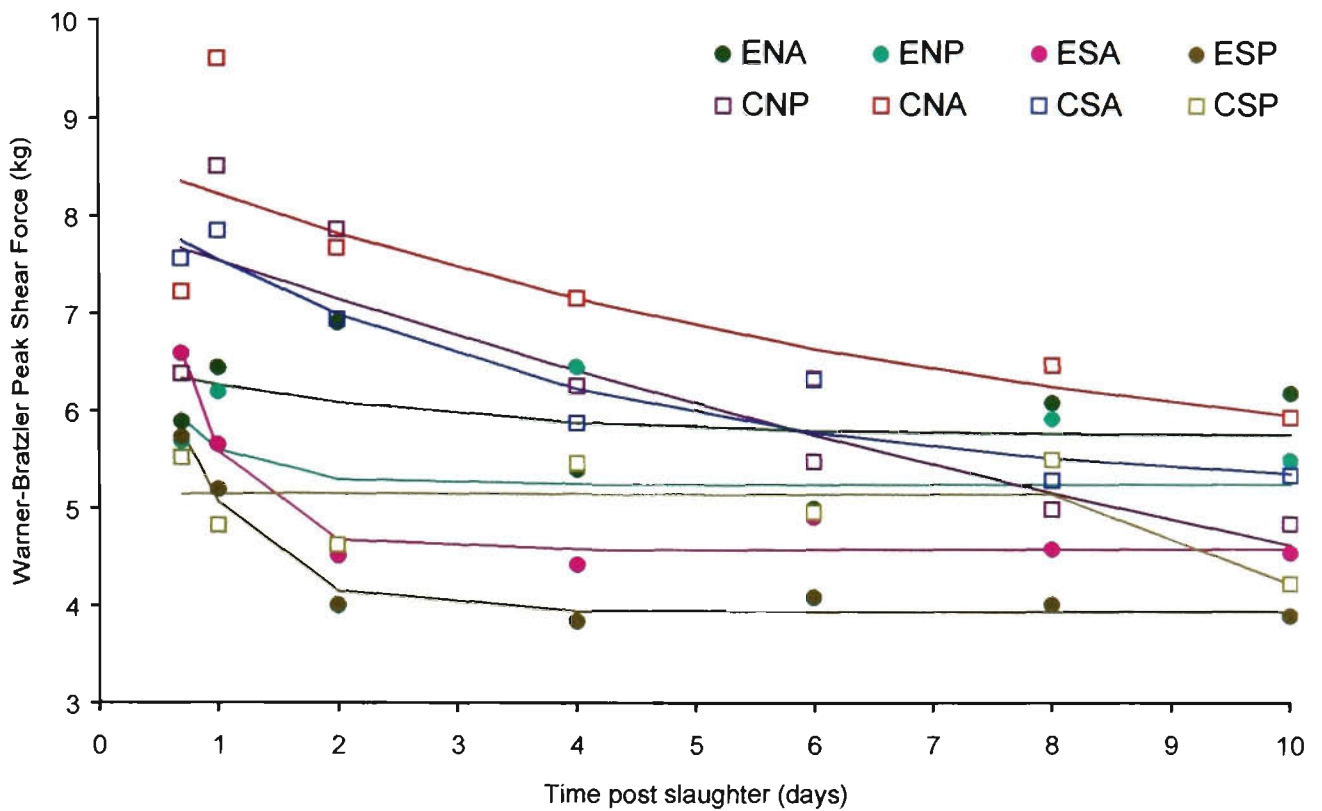


Figure 5.4 Mean Warner-Bratzler peak shear force (WBSF) in experiment 7 for pork *M. longissimus thoracis et lumborum* after carbon dioxide (C) or electrical head to heart stunning (E), low voltage electrical stimulation (none (N) or for 15 seconds 5 minutes post slaughter (S)) and pelvic (P) or Achilles tendon (A) suspension until rigor. Each point represents the actual WBSF at each time measured and the line represents the fitted curve.

The equations for each line are:

$$\text{WBSF}_{\text{CAN}} = 4.98 + 3.70 e^{-0.134t} \quad (R^2 = 43.3, \text{RSD} = 0.92),$$

$$\text{WBSF}_{\text{CNP}} = -0.4 + 8.4 e^{-0.05t} \quad (R^2 = 58.7, \text{RSD} = 0.90),$$

$$\text{WBSF}_{\text{CSA}} = 5.13 + 3.14 e^{-0.262t} \quad (R^2 = 86.1, \text{RSD} = 0.38),$$

$$\text{WBSF}_{\text{CSP}} = 5.145 - 0.93 \times 10^{-16} e^{+3.68t} \quad (R^2 = 22.9, \text{RSD} = 0.44),$$

$$\text{WBSF}_{\text{ENA}} = 5.74 + 0.8 e^{-0.43t} \quad (R^2 = 0, \text{RSD} = 0.72),$$

$$\text{WBSF}_{\text{ENP}} = 5.25 + 3.2 e^{-2.22t} \quad (R^2 = 0, \text{RSD} = 1.16),$$

$$\text{WBSF}_{\text{ESA}} = 4.58 + 10.67 e^{-2.37t} \quad (R^2 = 93.5, \text{RSD} = 0.21), \text{ and}$$

$$\text{WBSF}_{\text{ESP}} = 3.93 + 5.90 e^{-1.65t} \quad (R^2 = 96.4, \text{RSD} = 0.14).$$

The average sed = 1.08.

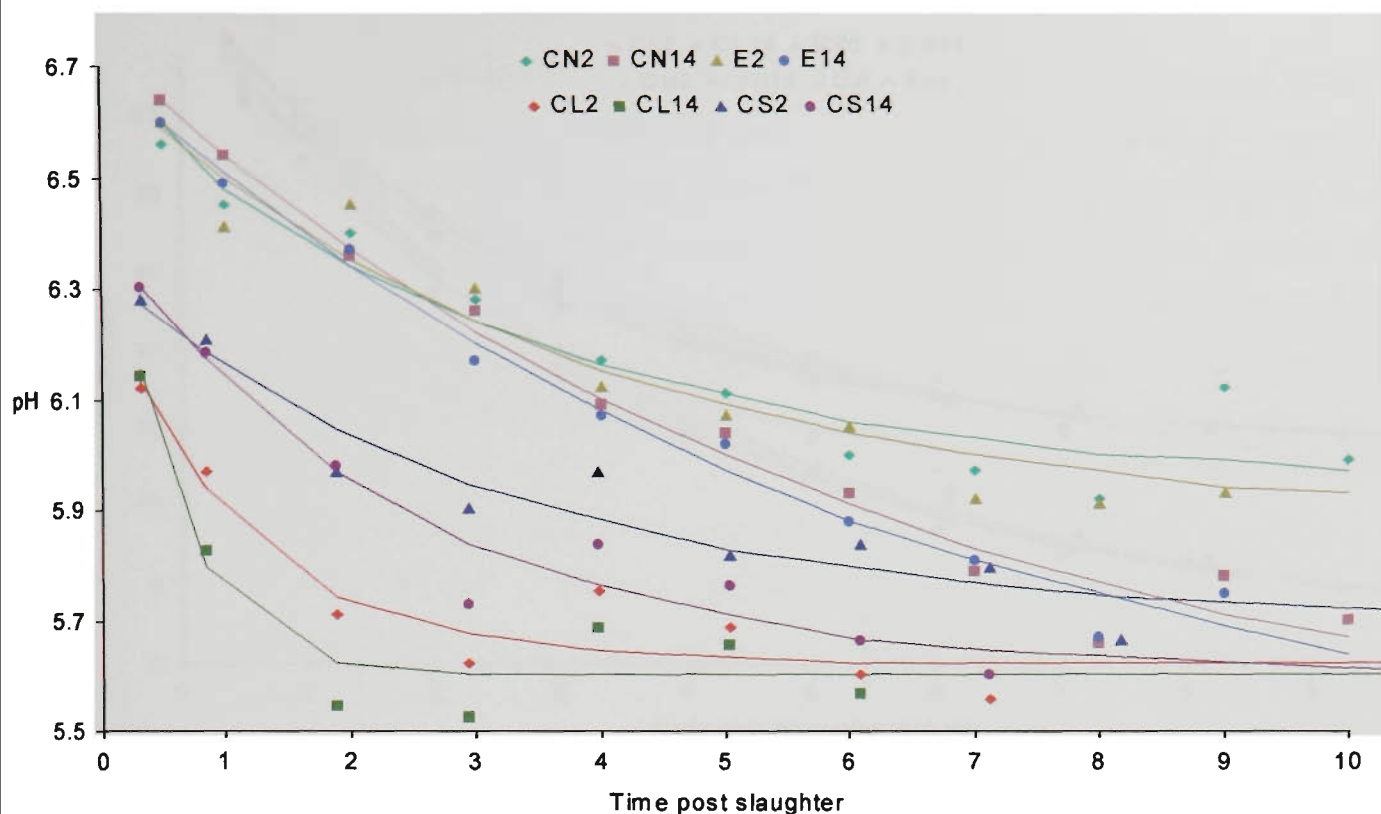


Figure 5.5 Mean rate of pH decline in experiment 8 for pork *M. longissimus thoracis et lumborum* after carbon dioxide (C) or electrical head to heart stunning (E), low voltage electrical stimulation (none (N), for 15 seconds (S) or for 60 seconds (L) 5 minutes post slaughter) and chilling at 2 or 14°C until rigor. Each point represents the actual pH at each time measured and the line represents the fitted curve.

The equations for each line are:

$$pH_{E2} = 5.88 + 0.81 e^{-0.27t}, (R^2 = 89.5, RSD = 0.08)$$

$$pH_{E14} = 5.38 + 1.32 e^{-0.16t}, (R^2 = 98.1, RSD = 0.04)$$

$$pH_{CN2} = 5.92 + 0.73 e^{-0.29t}, (R^2 = 89.7, RSD = 0.07)$$

$$pH_{CN14} = 5.42 + 1.33 e^{-0.17t}, (R^2 = 97.5, RSD = 0.05)$$

$$pH_{CS2} = 5.72 + 0.62 e^{-0.33t}, (R^2 = 88.6, RSD = 0.06)$$

$$pH_{CS14} = 5.64 + 0.81 e^{-0.44t}, (R^2 = 92.2, RSD = 0.06)$$

$$pH_{CL2} = 5.65 + 0.75 e^{-0.96t}, (R^2 = 86.7, RSD = 0.07)$$

$$pH_{CL14} = 5.63 + 1.43 e^{-2.05t}, (R^2 = 86.9, RSD = 0.07)$$

The average sed = 0.107.

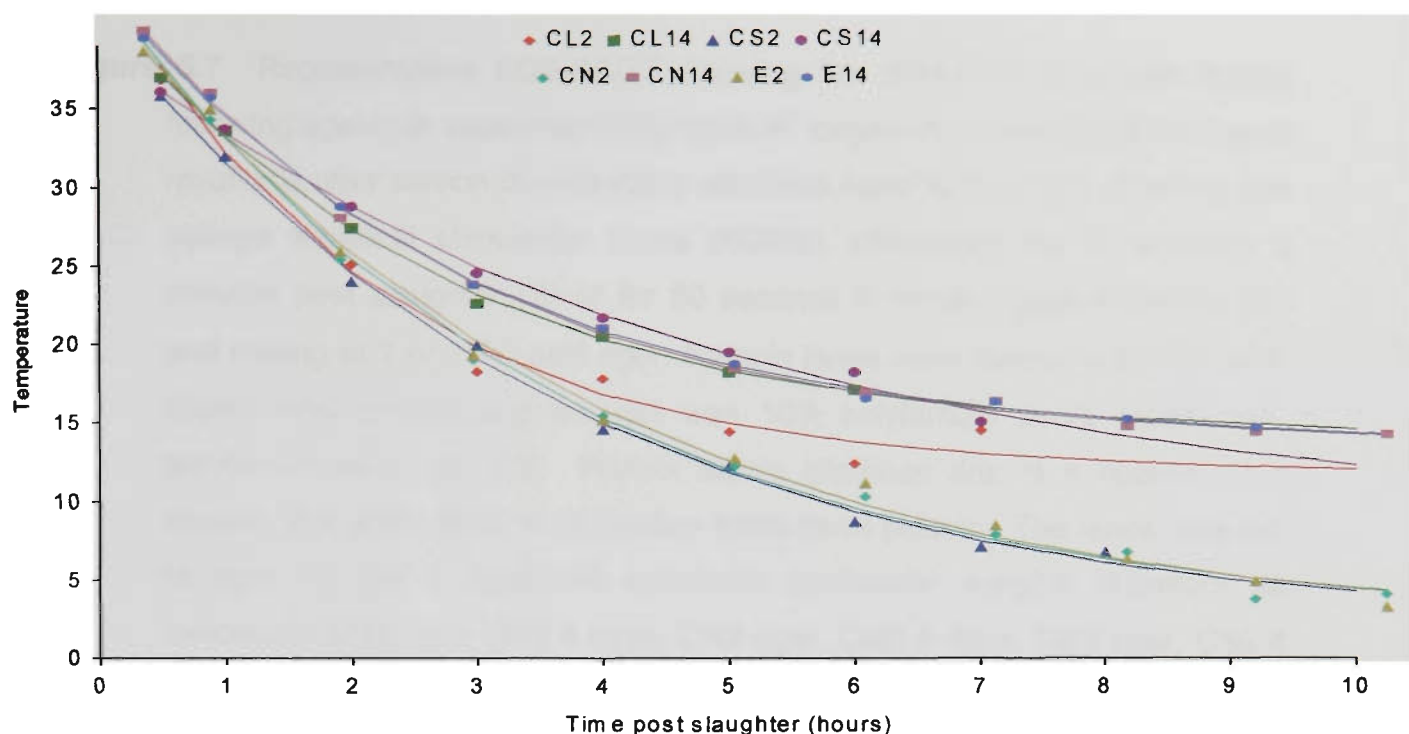


Figure 5.6 Mean rate of temperature decline (temp, °C) in experiment 8 for pork *M. longissimus thoracis et lumborum* after carbon dioxide (C) or electrical head to heart stunning (E), low voltage electrical stimulation (none (N), for 15 seconds (S) or for 60 seconds (L) 5 minutes post slaughter) and chilling at 2 or 14°C until rigor. Each point represents the actual temperature at each time measured and the line represents the fitted curve.

The equations for each line are:

$$\text{temp}_{\text{E2}} = 2.98 + 38.8 e^{-0.27t}, \quad (R^2 = 99.5, \text{RSD} = 0.79),$$

$$\text{temp}_{\text{E14}} = 13.89 + 28.47 e^{-0.36t}, \quad (R^2 = 99.9, \text{RSD} = 0.30),$$

$$\text{temp}_{\text{CN2}} = 3.62 + 38.98 e^{-0.30t}, \quad (R^2 = 99.6, \text{RSD} = 0.69),$$

$$\text{temp}_{\text{CN14}} = 13.88 + 29.02 e^{-0.37t}, \quad (R^2 = 99.8, \text{RSD} = 0.35),$$

$$\text{temp}_{\text{CS2}} = 1.56 + 39.43 e^{-0.27t}, \quad (R^2 = 99.6, \text{RSD} = 0.64),$$

$$\text{temp}_{\text{CS14}} = 8.61 + 30.68 e^{-0.21t}, \quad (R^2 = 99.5, \text{RSD} = 0.53),$$

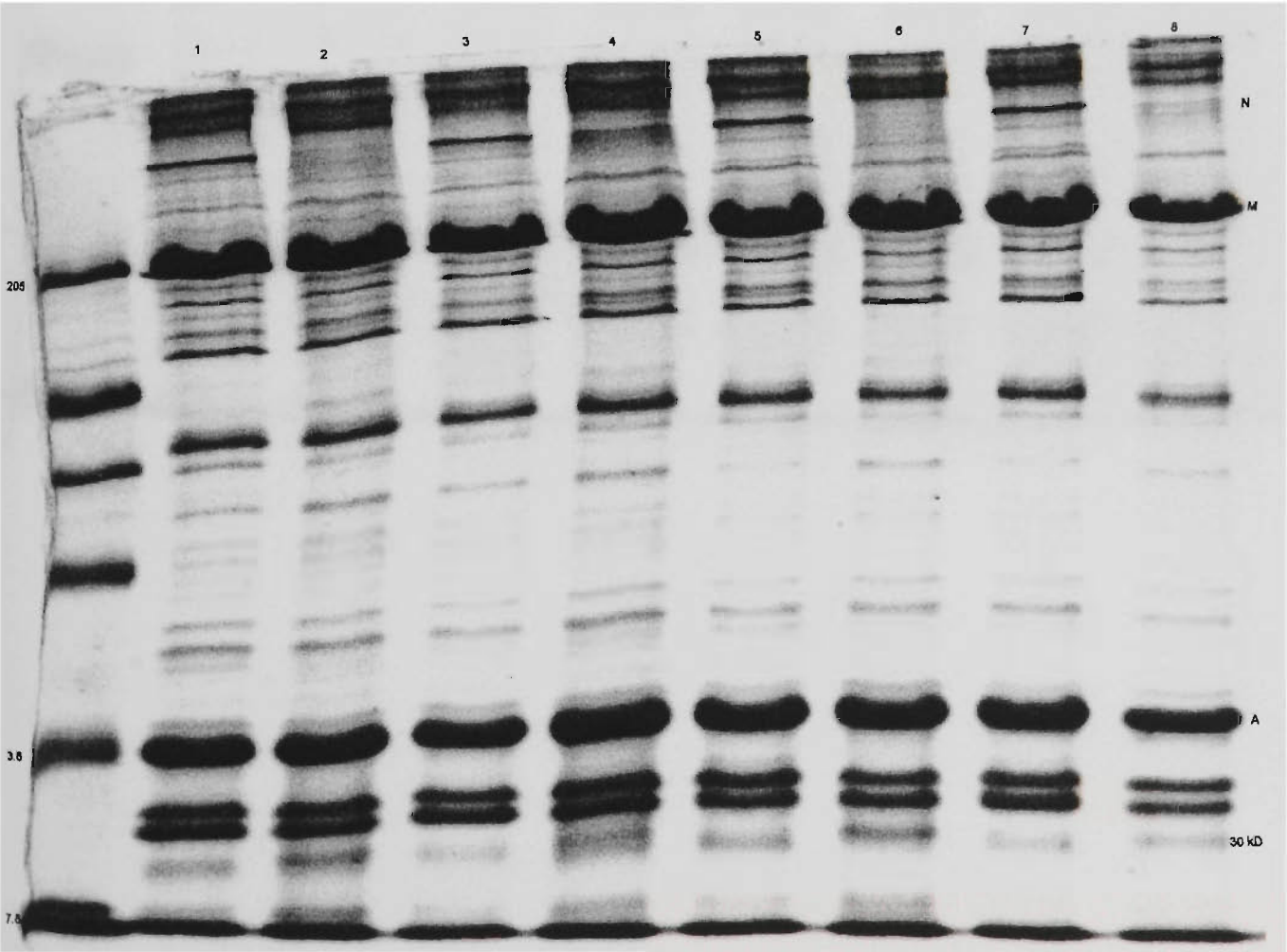
$$\text{temp}_{\text{CL2}} = 11.79 + 32.88 e^{-0.47t}, \quad (R^2 = 97.5, \text{RSD} = 1.46),$$

$$\text{temp}_{\text{CL14}} = 13.93 + 28.15 e^{-0.37t}. \quad (R^2 = 99.8, \text{RSD} = 0.35).$$

The average sed = 1.27.

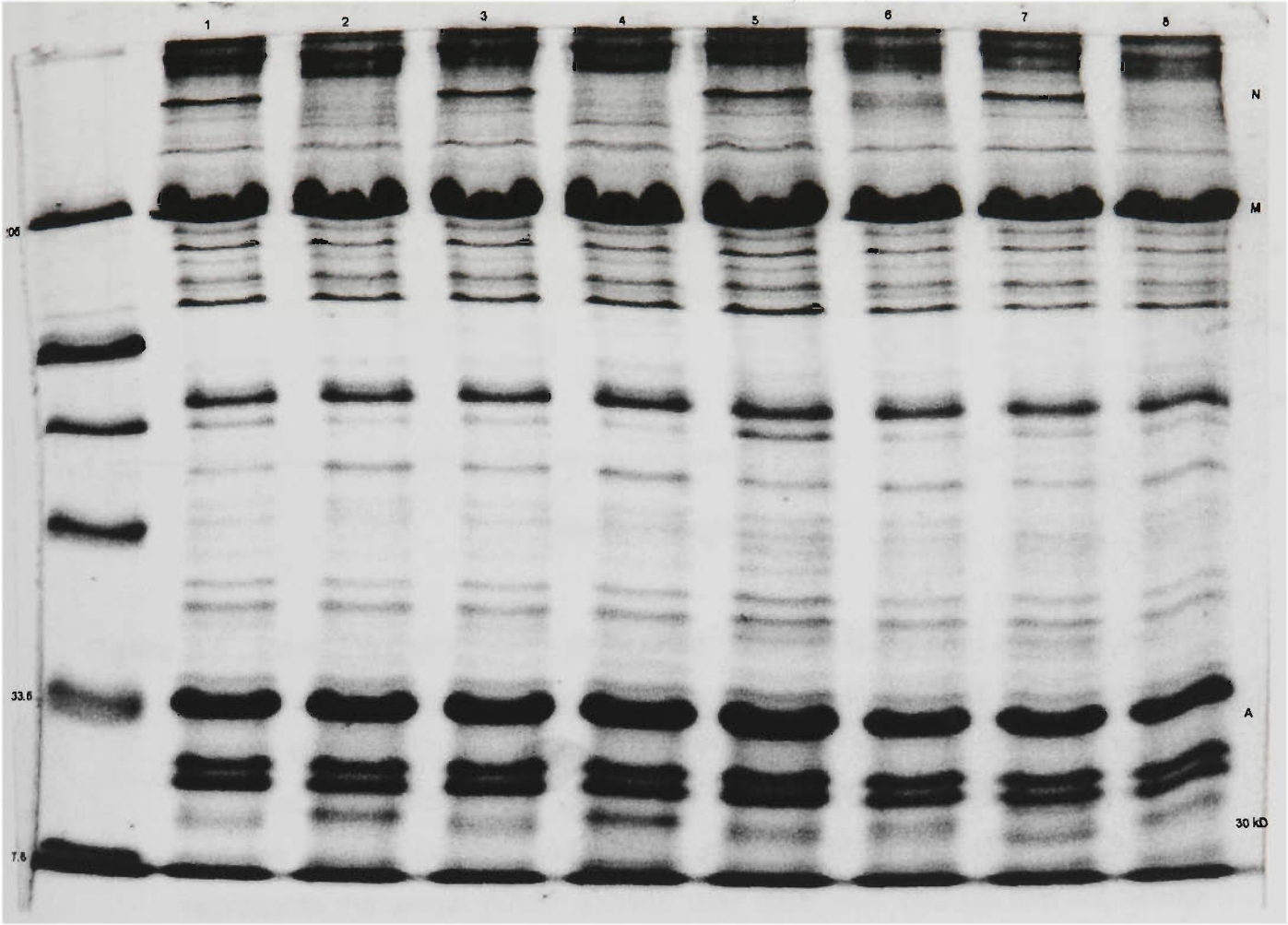
Figure 5.7 Representative SDS-PAGE showing the difference in protein bands following ageing in experiment 8 for pork *M. longissimus thoracis et lumborum* myofibrils after carbon dioxide (C) or electrical head to heart (E) stunning, low voltage electrical stimulation (none (NONE), stimulation for 15 seconds 5 minutes post slaughter (S) or for 60 seconds 5 minutes post slaughter (L)) and chilling at 2 or 14°C until rigor. Sample lanes were loaded with 10µl of 4 mg/ml total protein and the gel was 10% acrylamide (w/v), 0.06% bis-acrylamide (w/v), pH 9.3. Protein bands identified are; N = nebulin, M = myosin, A = actin, 30kD = 30 Kdalton breakdown protein. The lanes from left to right on gel 1 represent standards (molecular weights (kDalton) as indicated), EN2 rigor, EN2 4 days, CN2 rigor, CN2 4 days, CS2 rigor, CS2 4 days, CL2 rigor, CL2 4 days.

Gel 1



The lanes from left to right on gel 2 represent standards (molecular weights (kDaltons) as indicated), EN14 rigor, EN14 4 days, CN14 rigor, CN14 4 days, CS14 rigor, CS14 4 days, CL14 rigor, CL14 4 days.

Gel 2



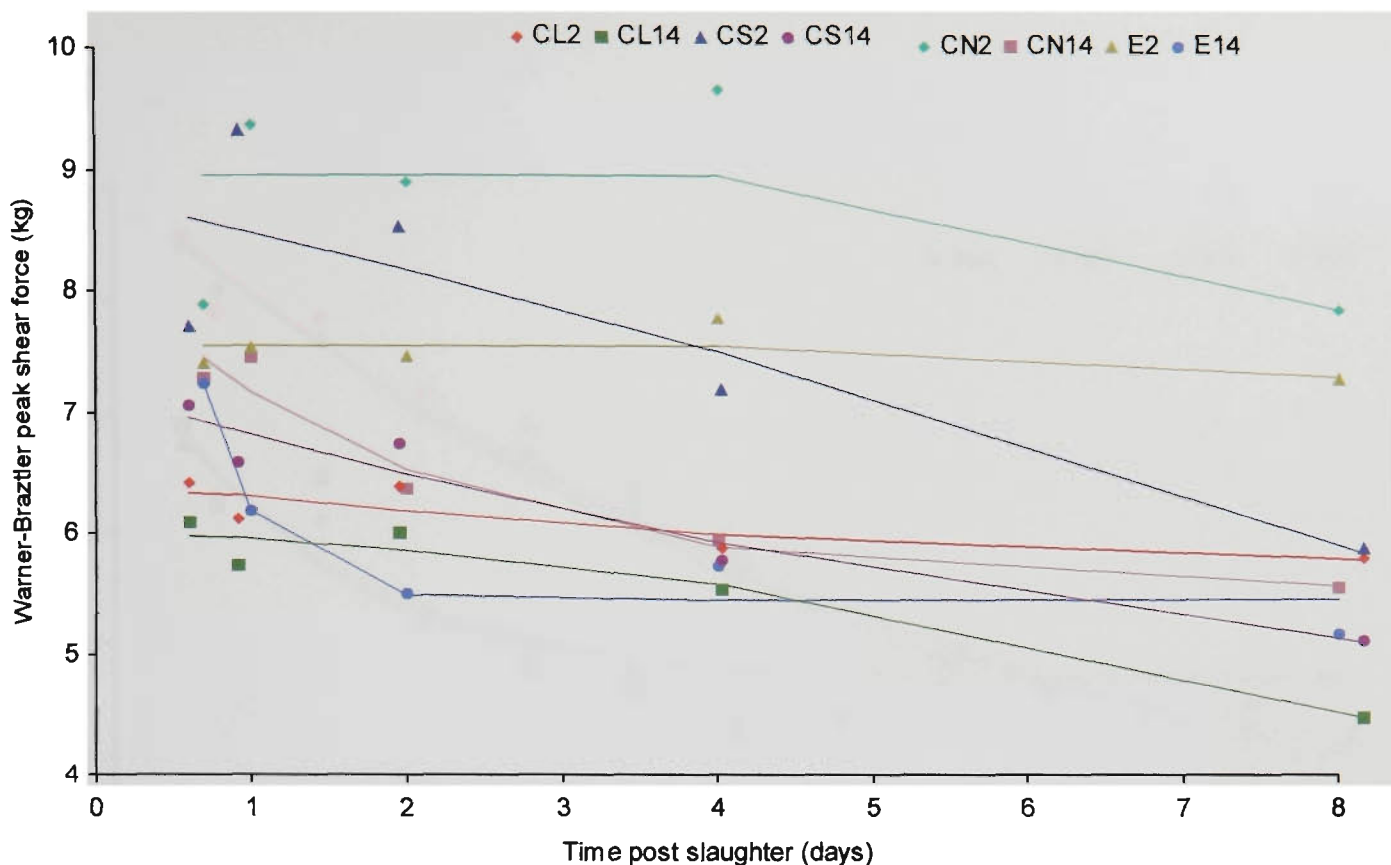


Figure 5.8 Mean Warner-Bratzler peak shear force (WBSF) over a 8 day ageing period in experiment 8 for pork *M. longissimus thoracis et lumborum* after carbon dioxide (C) or electrical head to heart stunning (E), low voltage electrical stimulation (none (N), for 15 seconds (S) or for 60 seconds (L) 5 minutes post slaughter) and chilling at 2 or 14°C until rigor. Each point represents the actual WBSF at each time measured and the line represents the fitted curve.

The equations for each line are:

$$\text{WBSF}_{\text{E2}} = 7.54 + 8.55 e^{-2.45t}, \quad (R^2 = 0, \text{RSD} = 0.20),$$

$$\text{WBSF}_{\text{E14}} = 5.85 + 9.31 e^{-1.34t}, \quad (R^2=87.8, \text{RSD} = 0.28),$$

$$\text{WBSF}_{\text{CN2}} = 9.21 + 3.73 e^{-2.11t}, \quad (R^2 = 0, \text{RSD} = 0.95),$$

$$\text{WBSF}_{\text{CN14}} = 5.51 + 2.73 e^{-0.49t}, \quad (R^2= 90.8, \text{RSD} = 0.25),$$

$$\text{WBSF}_{\text{CS2}} = 13.2 - 4.6 e^{+0.06t}, \quad (R^2 = 51.4, \text{RSD} = 0.85),$$

$$\text{WBSF}_{\text{CS14}} = 3.98 + 3.20 e^{-0.12t}, \quad (R^2=87.5, \text{RSD} = 0.26),$$

$$\text{WBSF}_{\text{CL2}} = 5.63 + 0.83 e^{-0.18t}, \quad (R^2 = 40.9, \text{RSD} = 0.20),$$

$$\text{WBSF}_{\text{CL14}} = 6.36 - 0.3 e^{+0.22t} \quad (R^2 = 89.7, \text{RSD} = 0.19).$$

The average sed = 0.99.

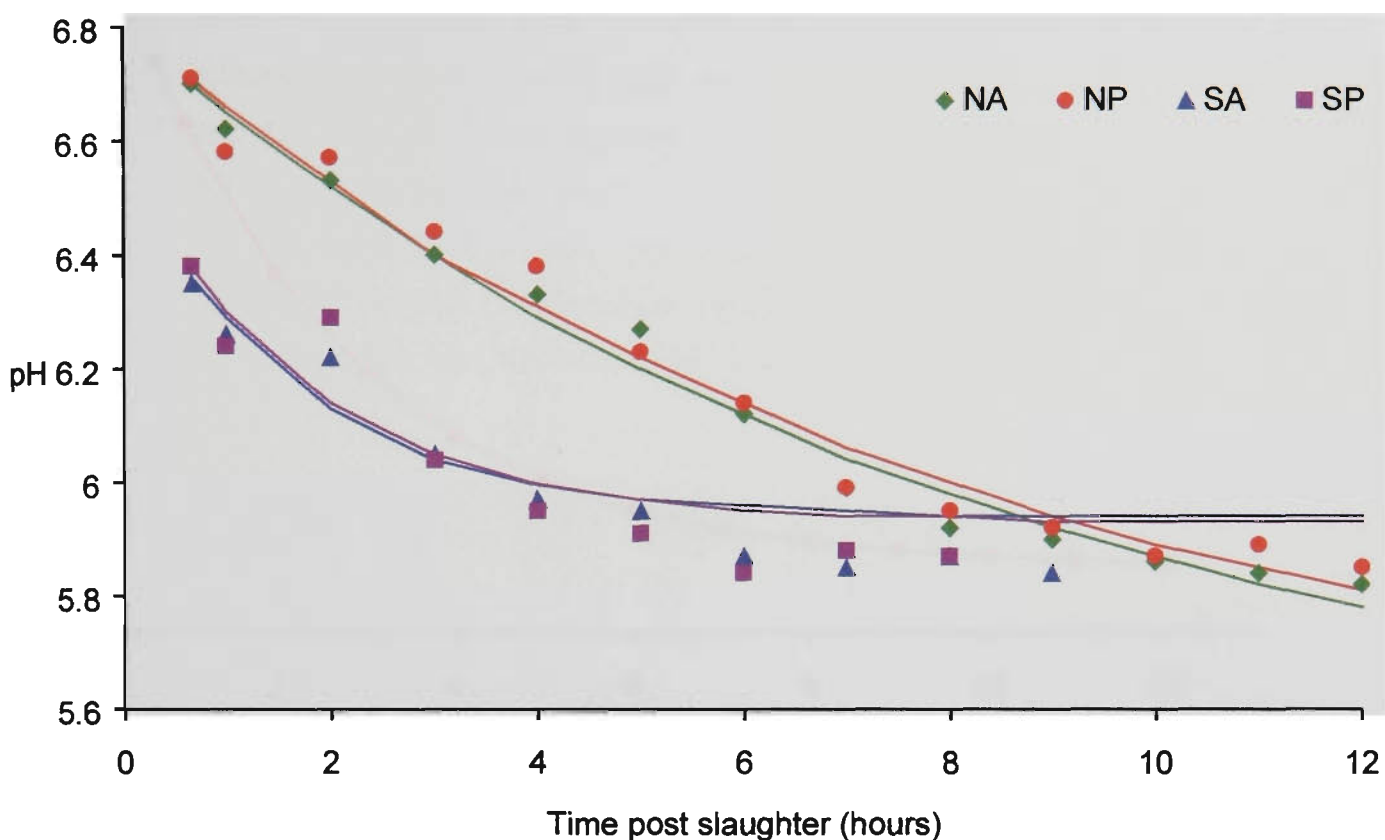


Figure 5.9 Mean rate of pH decline in experiment 9 for lamb *M. longissimus thoracis et lumborum* after low voltage electrical stimulation (none (N) or for 50 seconds at 5 minutes post slaughter (S)) and pelvic (P) or Achilles (A) suspension. Each point represents the actual pH at each time measured and the line represents the fitted curve.

The equations for each line are:

$$\text{pH}_{\text{SA}} = 5.94 + 0.63 e^{-0.60t} \quad (R^2 = 48.0, \text{RSD} = 0.195),$$

$$\text{pH}_{\text{SP}} = 5.93 + 0.66 e^{-0.57t} \quad (R^2 = 61.1, \text{RSD} = 0.119),$$

$$\text{pH}_{\text{NA}} = 5.46 + 1.35 e^{-0.12t} \quad (R^2 = 98.4, \text{RSD} = 0.04) \text{ and}$$

$$\text{pH}_{\text{NP}} = 5.50 + 1.31 e^{-0.12t} \quad (R^2 = 97.2, \text{RSD} = 0.05).$$

The average sed = 0.07.

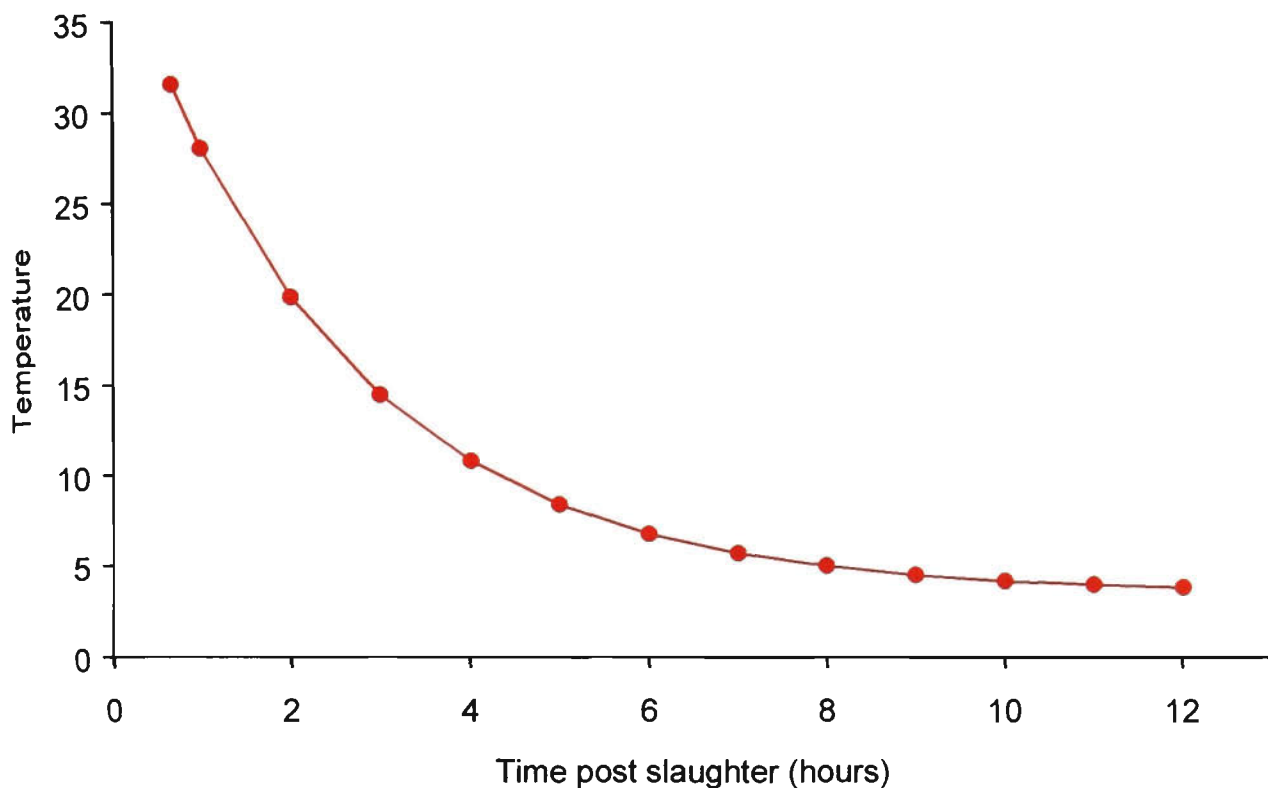


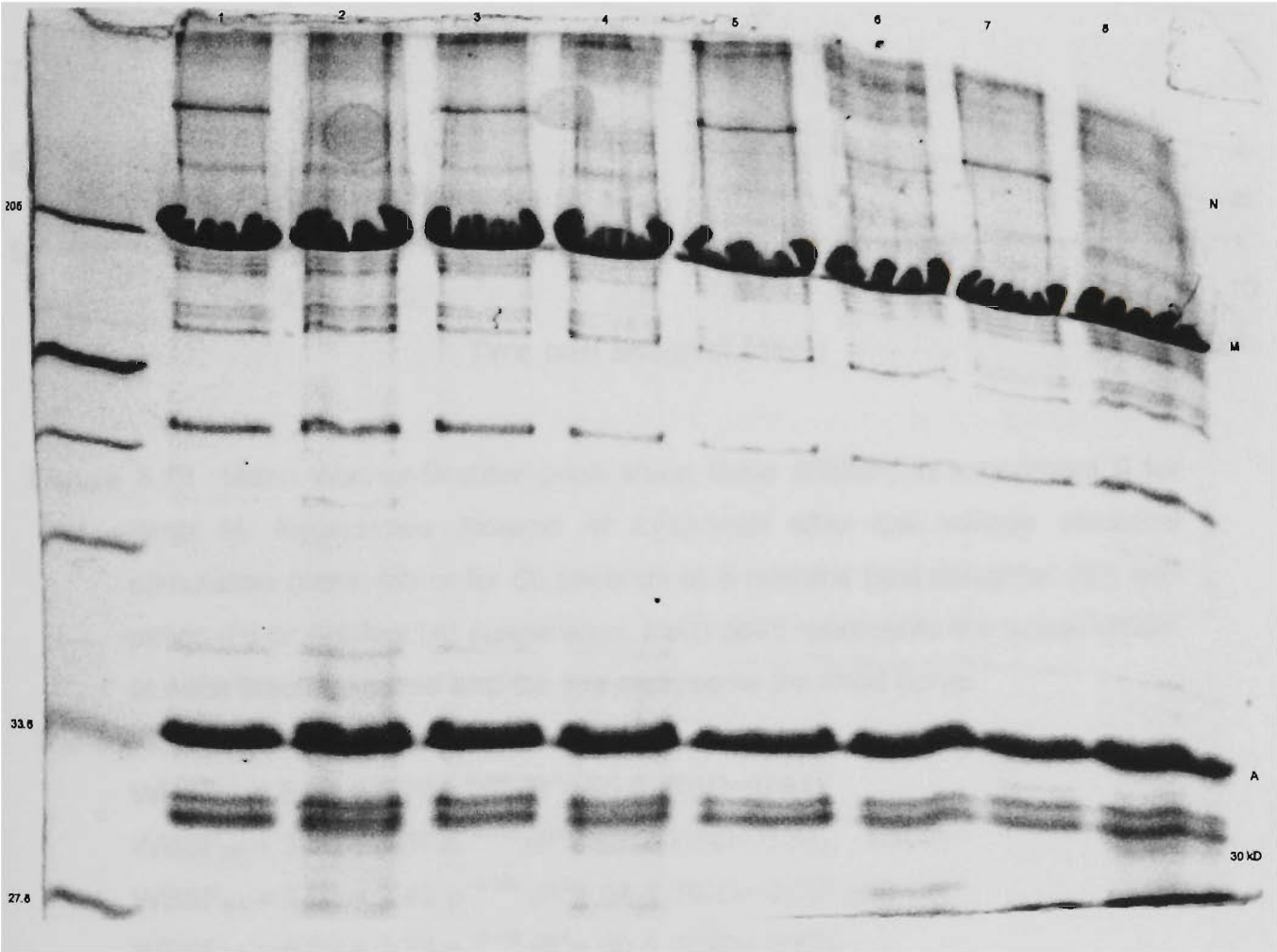
Figure 5.10 Mean rate of temperature decline (temp, °C) in experiment 9 for lamb *M. longissimus thoracis et lumborum* after low voltage electrical stimulation (none or for 50 seconds at 5 minutes post slaughter) and pelvic or Achilles suspension. Each point represents the actual temperature at each time measured and the line represents the fitted curve.

The equation for the line is:

$$\text{temp} = 3.553 + 36.65 e^{-0.40t} \quad (R^2=99.9, \text{RSD}=0.36).$$

The average sed = 0.75.

Figure 5.11 Representative SDS-PAGE showing the difference in protein bands following ageing in experiment 9 for lamb *M. longissimus thoracis et lumborum* myofibrils after low voltage electrical stimulation (none (N) or for 50 seconds 5 minutes post slaughter (S)) and pelvic (P) or Achilles (A) suspension. Sample lanes were loaded with 10 µl of 4 mg/ml total protein and the gel was 10% acrylamide (w/v), 0.06% bis-acrylamide (w/v), pH 9.3. Protein bands identified are; N = nebulin, M = myosin, A = actin, 30kD = 30 Kdalton breakdown protein. The lanes from left to right represent standards (molecular weights (kDaltons) as indicated), NA rigor, NA 4 days, NP rigor, NP 4 days, SA rigor, SA 4 days, SP rigor, SP 4 days.



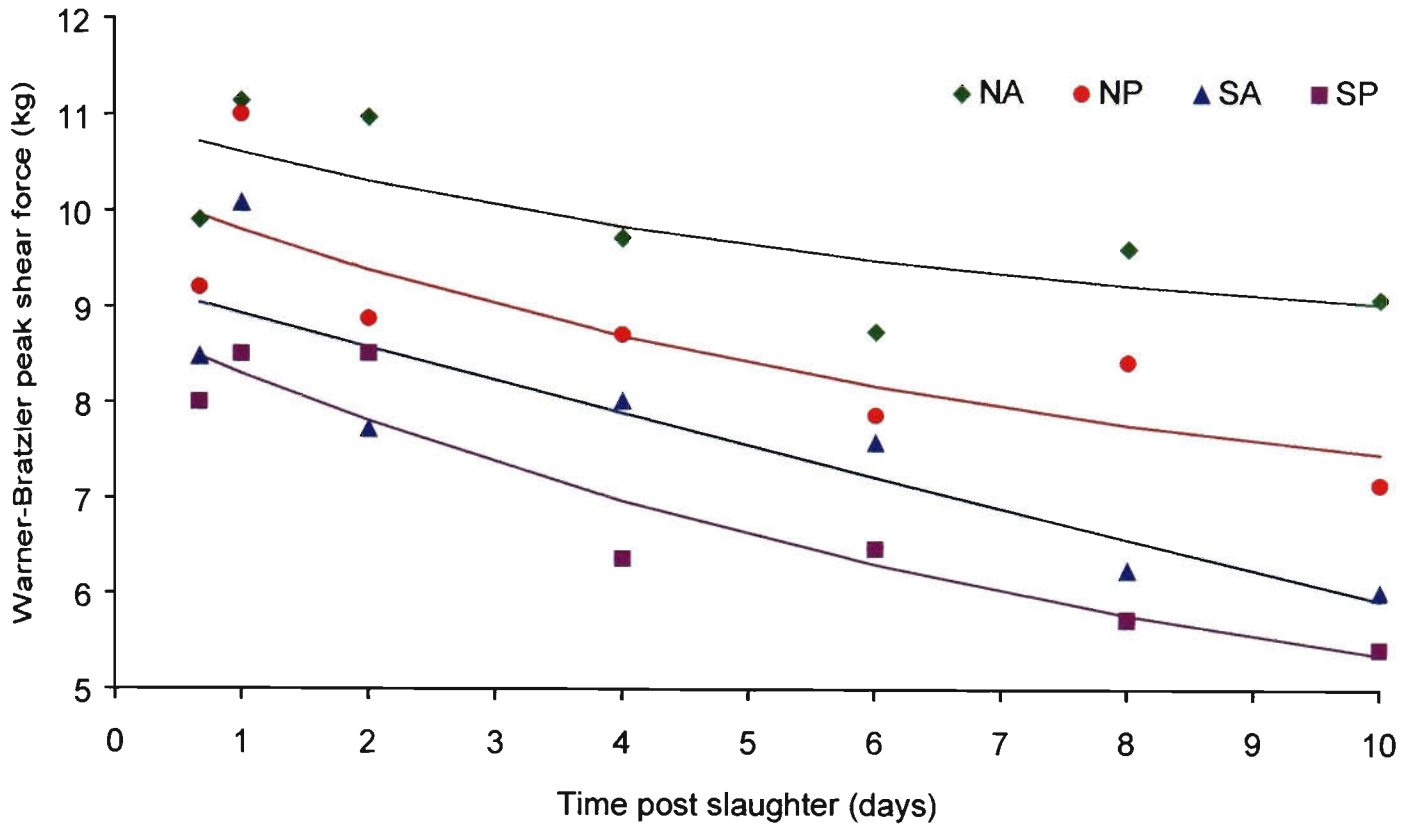


Figure 5.12 Mean Warner-Bratzler peak shear force (WBSF) in experiment 9 for lamb *M. longissimus thoracis et lumborum* after low voltage electrical stimulation (none (N) or for 50 seconds at 5 minutes post slaughter (S)) and pelvic (P) or Achilles (A) suspension. Each point represents the actual WBSF at each time measured and the line represents the fitted curve.

The equations for each line are:

$$\text{WBSF}_{\text{SA}} = 6.61 + 1.79 e^{-0.94t} \quad (R^2 = 65.2, \text{RSD} = 0.81),$$

$$\text{WBSF}_{\text{SP}} = 3.30 + 5.07 e^{-0.12t} \quad (R^2 = 83.2, \text{RSD} = 0.54),$$

$$\text{WBSF}_{\text{NA}} = 8.02 + 2.43 e^{-0.16t} \quad (R^2 = 34.4, \text{RSD} = 0.72) \text{ and}$$

$$\text{WBSF}_{\text{NP}} = 6.03 + 3.75 e^{-0.14t} \quad (R^2 = 50.6, \text{RSD} = 0.85).$$

The average sed = 0.83.

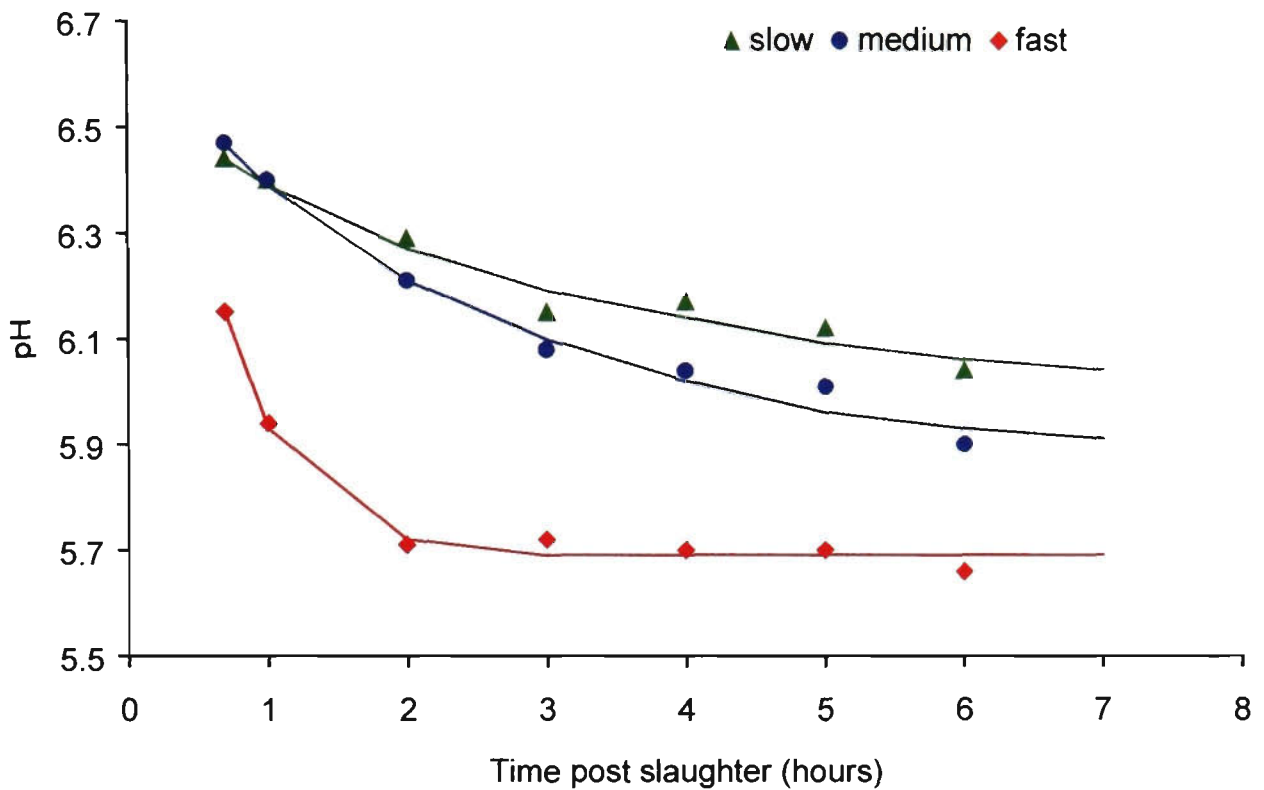


Figure 5.13 Mean rate of pH decline for pork *M. longissimus thoracis et lumborum* from experiments 7 and 8 grouped according to the rate of pH decline post slaughter (slow, medium and fast). Each point represents the actual pH at each time measured and the line represents the fitted curve.

The equations for each line are:

$$\text{pH}_{\text{slow}} = 5.99 + 0.57 e^{-0.34t}, (R^2 = 94.9, \text{RSD} = 0.03),$$

$$\text{pH}_{\text{medium}} = 5.87 + 0.80 e^{-0.41t}, (R^2 = 97.9, \text{RSD} = 0.03), \text{ and}$$

$$\text{pH}_{\text{fast}} = 5.69 + 1.99 e^{-2.09t} (R^2 = 98.6, \text{RSD} = 0.02).$$

The average sed = 0.06.

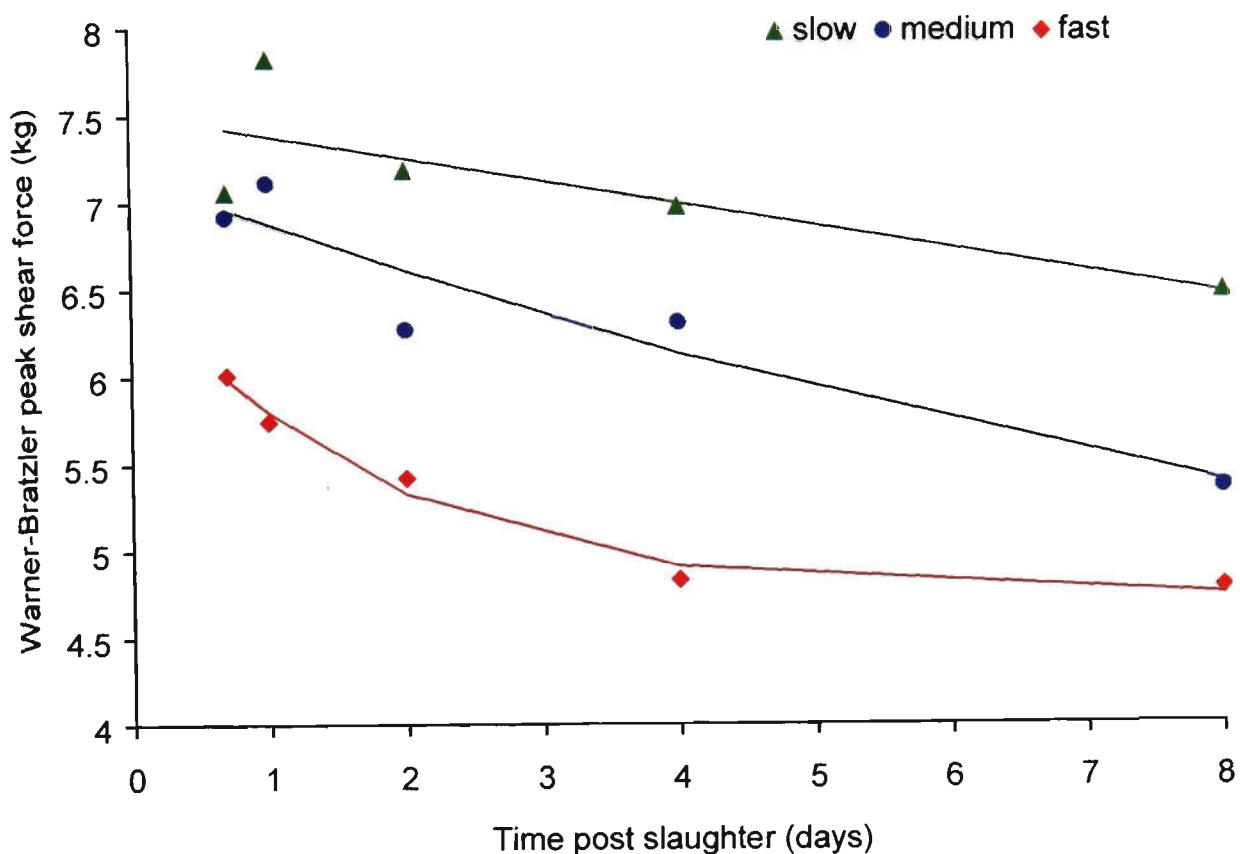


Figure 5.14 Mean Warner-Bratzler peak shear force (WBSF) for pork *M. longissimus thoracis et lumborum* from experiments 7 and 8 grouped according to the rate of pH decline post slaughter (slow, medium and fast) over an 8 day ageing period. Each point represents the actual WBSF at each time measured and the line represents the fitted curve.

The equations for each line are:

$$\text{WBSF}_{\text{slow}} = 22 - 14 e^{-0.01t}, (R^2 = 28.5, \text{RSD} = 0.41),$$

$$\text{WBSF}_{\text{medium}} = 3.59 + 3.58 e^{-0.08t}, (R^2 = 78.5, \text{RSD} = 0.31) \text{ and}$$

$$\text{WBSF}_{\text{fast}} = 4.72 + 1.89 e^{-0.56t}, (R^2 = 96.6, \text{RSD} = 0.10).$$

The average sed = 0.57.

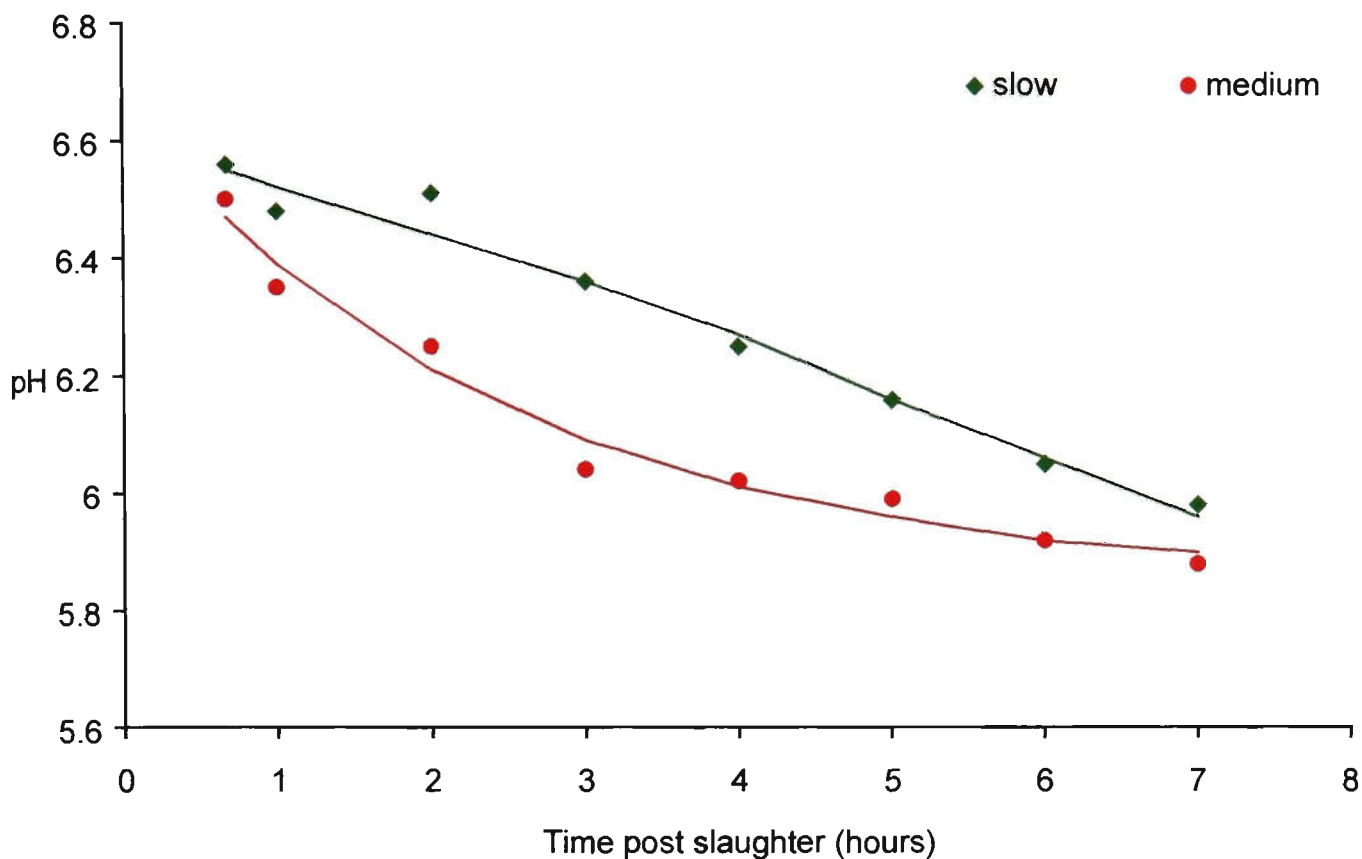


Figure 5.15 Mean rate of pH decline post slaughter for lamb *M. longissimus thoracis et lumborum* from experiment 9 grouped according to the rate of pH decline post slaughter (slow and medium). Each point represents the actual pH at each time measured and the line represents the fitted curve.

The equations for each line are:

$$\text{pH}_{\text{slow}} = 8.27 - 1.66 e^{-0.05t}, (R^2 = 96.8, \text{RSD} = 0.03)$$

$$\text{pH}_{\text{medium}} = 5.85 + 0.83 e^{-0.42t}, (R^2 = 96.5, \text{RSD} = 0.04).$$

The average sed = 0.07.

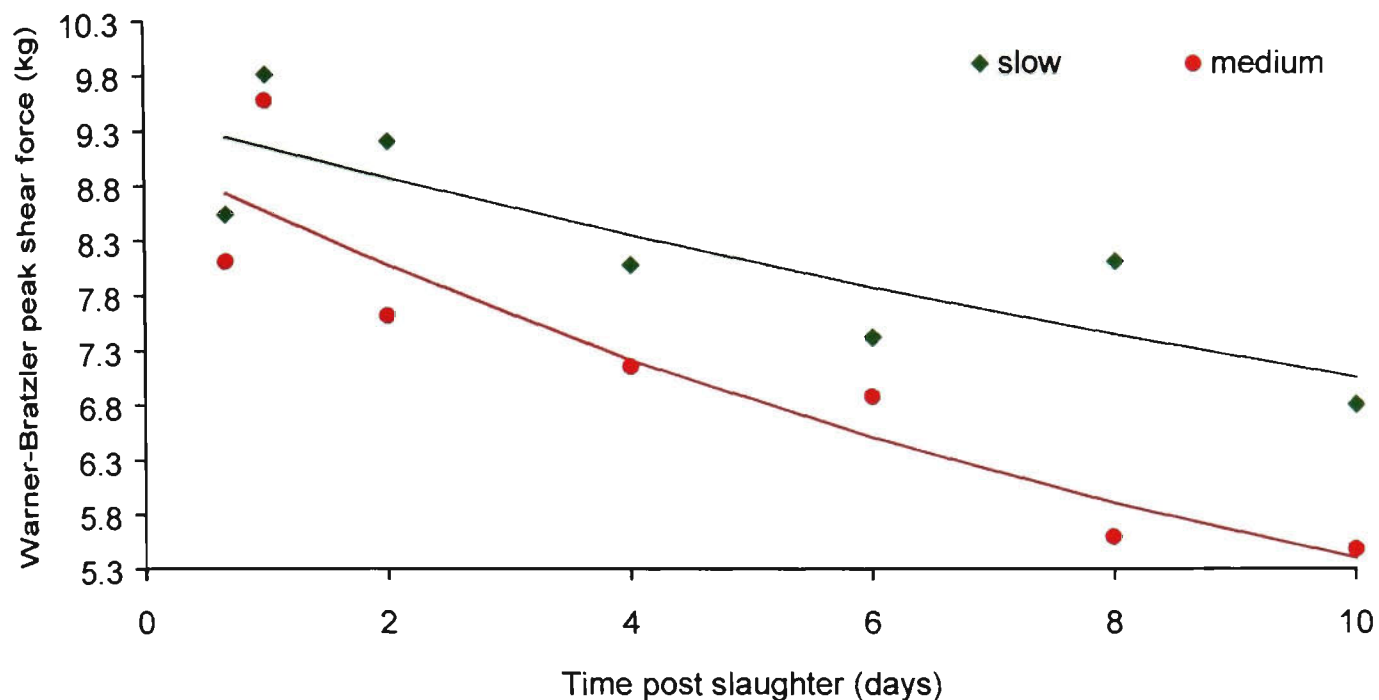


Figure 5.16 Mean Warner-Bratzler peak shear force (WBSF) for lamb *M. longissimus thoracis et lumborum* from experiment 9 grouped according to the rate of pH decline post slaughter (slow and medium). Each point represents the actual WBSF at each time measured and the line represents the fitted curve.

The equations for each line are:

$$\text{WBSF}_{\text{slow}} = 3.30 + 6.10 e^{-0.05t}, (R^2 = 56.4, \text{RSD} = 0.67)$$

$$\text{WBSF}_{\text{medium}} = 2.88 + 6.23 e^{-0.09t}, (R^2 = 77.6, \text{RSD} = 0.68).$$

The average sed = 0.07.

Table 5.1 Myofibrillar fragmentation index, percentage change in MFI, time to rigor and sarcomere length at rigor and 4 days post slaughter in experiment 7 for pork *M. longissimus thoracis et lumborum* after carbon dioxide or electrical head to heart stunning, low voltage electrical stimulation for 15 seconds or no electrical stimulation and pelvic or Achilles tendon suspension.

	Stunning ¹			Stimulation ²			Suspension ³			Significance ⁴		
	CO ₂	Elect	None	None	15 sec	Achilles	Pelvic	Stun	Stim	Susp	StimxSusp	SED
Time ⁵	8.25 ^a	6.58 ^b	9.42 ^a		5.42 ^b	7.50	7.33	P < 0.05	P < 0.001	NS	NS	0.777
S.L. ⁶ rigor	1.73	1.84	1.68 ^a		1.89 ^b	1.73	1.83	NS	P < 0.05	P = 0.06	P < 0.05	0.075
S.L. 4 days	1.78	1.91	1.74 ^a		1.95 ^b	1.78 ^a	1.91 ^b	P = 0.09	P < 0.01	P < 0.05	NS	0.067
MFI ⁷ rigor	41.7	51.1	41.9		50.9	47.7	45.1	NS	NS	NS	NS	6.63
MFI 4 days	78.4	91.2	89.4		80.2	92.2	77.4	NS	NS	NS	NS	8.39
% change MFI ⁸	36.3	36.2	48.1 ^a		24.4 ^b	39.5	33.0	NS	P < 0.05	NS	NS	10.41

¹Stunning method, CO₂ = 90% Carbon dioxide in air, exposure time of 1.8 minutes; Elect = Head to heart electrical stunning, 1.3 amps for 4 seconds

²15 sec = low voltage electrical stimulation for 15 seconds, 4 minutes post slaughter using rectal probe and stick wound clip, none = no stimulation

³Suspension = pelvic suspension or Achilles tendon suspension from 20 minutes post slaughter.

⁴Susp = suspension, Stim = stimulation, Stun = stunning method, NS = no significant differences, ^{ab} within rows and treatment combinations, means with different superscripts differ significantly (P < 0.05), No other interactions occurred other than those presented in the table

⁵Time = time (hours) taken to reach rigor, defined as pH < 5.8

⁶S.L. = sarcomere length (µm)

⁷MFI = myofibrillar fragmentation index

⁸%change MFI = percentage increase in MFI from rigor to 4 days post slaughter

Table 5.2 Sarcoplasmic and myofibrillar protein solubility (mg/g) at 1, 3 and 5 hours post slaughter and rigor in experiment 7 for pork *M. longissimus thoracis et lumborum* after carbon dioxide or electrical head to heart stunning, low voltage electrical stimulation for 15 seconds or no electrical stimulation and pelvic or Achilles tendon suspension until rigor.

	Stunning ¹		Stimulation ²		Suspension ³			Significance ⁴			
	CO ₂	Elect	None	15 sec	Achilles	Pelvic	Stun	Stim	Susp	StimxSusp	SED
Sarcoplasmic protein solubility											
1 hour	29.8	36.5	32.7	33.5	34.5	31.7	NS	NS	NS	NS	5.75
3 hours	42.4	45.6	46.6	41.4	41.6	46.3	NS	NS	NS	NS	10.66
5 hours	31.4	30.8	34.5	27.7	26.9	35.4	NS	NS	NS	NS	8.21
Rigor ⁵	36.5	32.1	33.6	35.0	32.7	36.0	NS	NS	NS	NS	5.96
Myofibrillar protein solubility											
1 hour	205.0	189.4	201.2	193.1	202.7	191.6	NS	NS	NS	NS	13.16
3 hours	201.3	196.3	207.8	189.9	203.5	194.2	NS	NS	NS	NS	15.19
5 hours	185.1	204.2	187.1	202.1	199.8	189.5	NS	NS	NS	NS	17.85
rigor	213.4	237.6	226.8	224.2	221.0	230.0	P < 0.05	NS	NS	NS	16.18

¹Stunning method, CO₂ = 90% Carbon dioxide in air, exposure time of 1.8 minutes; Elect = Head to heart electrical stunning, 1.3 amps for 4 seconds

²15 sec = low voltage electrical stimulation for 15 seconds, 4 minutes post slaughter using rectal probe and stick wound clip, none = no stimulation

³Suspension = pelvic suspension or Achilles tendon suspension from 20 minutes post slaughter.

⁴Susp = suspension, Stim = stimulation, Stun = stunning method, NS = no significant differences,^{ab} within rows and treatment combinations, means with different superscripts differ significantly (P < 0.05). No other interactions occurred other than those presented in the table

⁵rigor = 1 hour post rigor defined as pH < 5.8

Table 5.3 Warner-Bratzler shear force (WBSF, kg) measurements at rigor, 1, 2, 4, 6, 8 and 10 days post slaughter and percentage change in WBSF in experiment 7 for pork *M. longissimus thoracis et lumborum* after carbon dioxide or electrical head to heart stunning, low voltage electrical stimulation for 15 seconds or no electrical stimulation and pelvic or Achilles tendon suspension.

	Stunning ¹			Stimulation ²			Suspension ³			Significance ⁴		
	CO ₂	Elect	None	None	15 sec	Achilles	Pelvic	Stun	Stim	Susp	StimxStun xSusp	SED
Rigor	6.67	5.97	6.29	6.35	6.35	6.81 ^a	5.83 ^b	NS	NS	P<0.001	NS	0.457
1 day	7.69 ^a	5.87 ^b	7.68 ^a	5.87 ^b	5.87 ^b	7.38 ^a	6.17 ^b	P < 0.05	P < 0.05	P < 0.01	NS	0.689
2 days	6.77 ^a	4.85 ^b	6.60 ^a	5.02 ^b	5.02 ^b	6.50 ^a	5.12 ^b	P < 0.05	P < 0.05	P < 0.001	P < 0.001	0.755
4 days	6.19	5.02	6.31	4.90	4.90	5.71	5.50	NS	NS	NS	NS	0.730
6 days	5.78	4.52	5.23	5.07	5.07	5.64 ^a	4.65 ^b	NS	NS	P < 0.01	NS	0.707
8 days	5.56	5.15	5.86	4.84	4.84	5.60	5.11	NS	NS	NS	NS	0.691
10 days	5.08	5.03	5.61 ^a	4.50 ^b	4.50 ^b	5.50	4.61	NS	P < 0.05	NS	NS	0.454
% change WBSF ⁵	5.0	14.9	-1.0 ^a	20.9 ^b	20.9 ^b	15.9	4.0	NS	P < 0.05	NS	NS	10.51

¹Stunning method, CO₂ = 90% Carbon dioxide in air, exposure time of 1.8 minutes; Elect = Head to heart electrical stunning, 1.3 amps for 4 seconds

²15 sec = low voltage electrical stimulation for 15 seconds, 4 minutes post slaughter using rectal probe and stick wound clip, none = no stimulation

³Suspension = pelvic suspension or Achilles tendon suspension from 20 minutes post slaughter.

⁴Susp = suspension, Stim = stimulation, Stun = stunning method, NS = no significant differences,^{ab} within rows and treatment combinations, means with different superscripts differ significantly (P < 0.05). No other interactions occurred other than those presented in the table

⁵% change WBSF = percentage change in WBSF from rigor to 4 days post slaughter

Table 5.4 Meat quality at rigor and 4 days post slaughter and pH at 3 hours post slaughter in experiment 7 for pork*M. longissimus thoracis et lumborum* after carbon dioxide or electrical head to heart stunning, low voltage electrical stimulation for 15 seconds or no electrical stimulation.

	Stunning ¹			Stimulation ²		Significance ³	
	CO ₂	Elect	None	15 sec	Stun	Stim	SED
pH 3 hours	6.11 ^a	5.89 ^b	6.20 ^a	5.80 ^b	P < 0.01	P < 0.001	0.076
Rigor							
pH	5.85 ^a	5.74 ^b	5.91 ^a	5.63 ^b	P = 0.06	P < 0.001	0.058
drip loss (%)	2.38 ^a	4.61 ^b	3.04	3.95	P < 0.05	NS	0.832
L*	43.8	46.4	43.5 ^a	46.7 ^b	P = 0.07	P < 0.05	1.33
a*	4.5	5.1	4.7	4.9	P = 0.06	NS	0.34
b*	2.3	2.7	2.3	2.7	NS	NS	0.28
4 days							
pH	5.41	5.38	5.41	5.38	NS	NS	0.024
L*	49.26	50.35	48.35 ^a	51.26 ^b	NS	P < 0.001	0.720
a*	5.2 ^a	6.6 ^b	5.6	6.2	P < 0.01	NS	0.44
b*	3.1 ^a	4.0 ^b	3.2	3.9	P < 0.05	P = 0.08	0.40

¹CO₂ = 90% Carbon dioxide in air, exposure time of 1.8 minutes; Elect = Head to heart electrical stunning, 1.3 amps for 4 seconds

²15 sec = low voltage electrical stimulation for 15 seconds, 4 minutes post slaughter using rectal probe and stick wound clip, none = no stimulation.

³Stim = stimulation, Stun = stunning method, NS = no significant differences,^{ab} within rows and treatment combinations, means with different superscripts differ significantly (P < 0.05). No interactions occurred other than those presented in the table

Table 5.5 Cooking loss at rigor, 1, 2, 4, 6, 8 and 10 days post slaughter and purge at 4 and 10 days post slaughter in experiment 7 for pork *M. longissimus thoracis et lumborum* after carbon dioxide or electrical head to heart stunning, low voltage electrical stimulation for 15 seconds or no electrical stimulation.

	Stunning ¹		Stimulation ²		Significance ³		
	CO ₂	Elect	None	15 sec	Stun	Stim	SED
Cooking loss (%)							
rigor	30.31	31.64	28.69 ^a	33.27 ^b	NS	P < 0.001	1.014
1 day	33.36	33.37	33.10	33.63	NS	NS	1.059
2 days	28.38	28.03	28.48	27.94	NS	NS	1.018
4 days	29.52 ^a	27.51 ^b	28.76	28.27	P < 0.05	NS	0.853
6 days	33.64 ^a	31.20 ^b	32.15	32.68	P < 0.05	NS	0.730
8 days	33.92	33.34	34.40	32.86	NS	NS	0.650
10 days	33.30	33.61	33.31	33.60	NS	NS	0.780
Purge (%)							
4 days	4.59 ^a	7.48 ^b	4.37 ^a	7.70 ^b	P < 0.01	P < 0.01	0.987
10 days	6.35 ^a	8.97 ^b	6.03 ^a	9.29 ^b	P < 0.01	P < 0.01	0.951

¹Stunning method, CO₂ = 90% Carbon dioxide in air, exposure time of 1.8 minutes; Elect = Head to heart electrical stunning, 1.3 amps for 4 seconds
²15 sec = low voltage electrical stimulation for 15 seconds, 4 minutes post slaughter using rectal probe and stick wound clip, none = no stimulation.

³Stim = stimulation, Stun = stunning method, NS = no significant differences,
^{ab} within rows and treatment combinations, means with different superscripts differ significantly (P < 0.05), No interactions occurred other than those presented in the table

Table 5.6 Myofibrillar fragmentation index, sarcoplasmic protein solubility, time to rigor and sarcomere length measurements at rigor and 4 days post slaughter in experiment 8 for pork *M. longissimus thoracis et lumborum* after carbon dioxide or electrical head to heart stunning, low voltage electrical stimulation for 15 seconds, 60 seconds or no low voltage electrical stimulation and chilling at 2 or 14°C until rigor.

	Stunning ¹ x Stimulation ²				Chilling ³				Stunning x Chilling				Significance		
	Elect	CO ₂ -None	CO ₂ -S	CO ₂ -L	2°C	14°C	CO ₂ -2°C	CO ₂ -14°C	CO ₂ -14°C	Elect-2°C	Elect-14°C	Stunning x Chilling	Stunning x Stimulation	Chilling	SED
time to rigor (hr)	9.2 ^c	8.33 ^c	5.8 ^b	3.2 ^a	7.2 ^a	5.9 ^b	7.0	5.7	5.7	7.7	6.6	NS	P < 0.001	P < 0.01	0.74
Sarc. protein ⁶	55.1	55.2	55.1	55.2	54.4	56.0	53.9	56.4	56.4	55.6	54.6	NS	NS	NS	2.69
S.L. ⁷ rigor	1.81 ^b	1.57 ^a	1.73 ^{ab}	1.88 ^b	1.71	1.79	1.73 ^a	1.72 ^a	1.72 ^a	1.62 ^a	1.99 ^b	P < 0.05	P < 0.05	NS	0.093
S.L. 4 days	1.83 ^b	1.64 ^a	1.88 ^b	1.88 ^b	1.76 ^a	1.86 ^b	1.75	1.85	1.85	1.77	1.89	NS	P < 0.01	P < 0.05	0.081
MFI ⁸ rigor	49.3	40.9	39.0	41.4	42.3	43.0	41.4	39.5	39.5	45.0	53.7	NS	NS	NS	8.93
MFI 4 days	73.1	64.2	65.2	67.7	56.9 ^a	78.2 ^b	56.0	75.5	75.5	59.6	86.5	NS	NS	P < 0.01	9.13
% change MFI ⁹	33.5	33.3	24.7	40.3	23.5 ^a	43.3 ^b	22.7	44.0	44.0	25.8	41.3	NS	NS	P < 0.05	9.92

¹CO₂ = 90% Carbon dioxide in air, exposure time of 1.8 minutes; Elect = Head to heart electrical stunning, 1.3 amps for 4 seconds

²S = low voltage electrical stimulation for 15 seconds, 4 minutes post slaughter using rectal probe and stick wound clip, L = = low voltage electrical stimulation for 60 seconds, 4 minutes post slaughter using rectal probe and stick wound clip, none = no stimulation.

³Chilling = sides chilled at either 2 or 14°C until a pH < 5.8 obtained then chilled at 2°C.

⁴NS= no significant differences, ^{abc} within rows and treatment combinations, means with different superscripts differ significantly (P < 0.05)

⁵rigor = 1 hour post rigor defined as pH < 5.8

⁶sarc. protein = sarcoplasmic protein solubility (mg/g)

⁷S.L. = sarcomere length (µm),

⁸MFI = myofibrillar fragmentation index,

⁹%change in MFI = percentage change in MFI from rigor to 4 days post slaughter

Table 5.7 Warner-Bratzler shear force (WBSF, kg) at rigor, 1, 2, 4 and 8 days post slaughter and percentage change in WBSF in experiment 8 for pork *M. longissimus thoracis et lumborum* after carbon dioxide or electrical head to heart stunning, low voltage electrical stimulation for 15 seconds, 60 seconds or no low voltage electrical stimulation and chilling at 2 or 14°C until rigor.

	Stunning ¹ x Stimulation ²				Chilling ³		Significance ⁴		SED
	Elect	CO ₂ - None	CO ₂ - S	CO ₂ - L	2°C	14°C	Stunning x Stimulation	Chilling	
rate ⁵	1.90	0.72	0.44	-0.30	1.11	0.27	NS	NS	0.843
rigor ⁶	7.33	7.58	7.31	6.27	7.26	6.99	NS	NS	0.747
1 day	6.86 ^{ab}	8.40 ^b	7.84 ^{ab}	5.96 ^a	8.04 ^a	6.50 ^b	P < 0.05	P < 0.001	0.994
2 days	6.48	7.63	7.55	6.21	7.79 ^a	6.14 ^b	NS	P < 0.01	0.461
4 days	6.75 ^{ab}	7.81 ^b	6.49 ^a	5.76 ^a	7.63 ^a	5.77 ^b	P < 0.05	P < 0.001	0.598
8 days	6.22	6.70	5.56	5.23	6.75 ^a	5.10 ^b	NS	P < 0.01	0.552
% Change WBSF ⁷	10.9	-2.6	7.9	6.0	-9.9 ^a	14.3 ^b	NS	P < 0.05	10.78

¹ CO₂ = 90% Carbon dioxide in air, exposure time of 1.8 minutes; Elect = Head to heart electrical stunning, 1.3 amps for 4 seconds

²S = low voltage electrical stimulation for 15 seconds, 4 minutes post slaughter using rectal probe and stick wound clip, L = low voltage electrical stimulation for 60 seconds, 4 minutes post slaughter using rectal probe and stick wound clip, none = no stimulation.

³Chilling = sides chilled at either 2 or 14°C until a pH < 5.8 obtained then chilled at 2°C.

⁴NS = no significant difference, ^{ab} within rows and treatment combinations, means with different superscripts differ significantly (P<0.05),

⁵rate = rate of ageing from rigor to 8 days post slaughter as determined by fitting an exponential decay equation

⁶rigor = 1 hour post rigor defined as pH < 5.8

⁷% change in WBSF = percentage change in WBSF from rigor to 4 days post slaughter

Table 5.8 Taste panel response for tenderness, juiciness, flavour and overall acceptability at 2 days post slaughter in experiment 8 for pork *M. longissimus thoracis et lumborum* after carbon dioxide or electrical head to heart stunning, low voltage electrical stimulation for 15 seconds, 60 seconds or no low voltage electrical stimulation and chilling at 2 or 14°C.

	Stunning ¹ x Stimulation ²				Chilling ³				Stunning x Chilling				Significance ⁴		
	Elect	CO ₂ - None	CO ₂ - S	CO ₂ - L	2°C	14°C	CO ₂ - 2°C	CO ₂ - 14°C	Elect- 2°C	Elect- 14°C	Stunning x Chilling	Stunning Stimulation	Chilling	SED	
tenderness ⁵	59.2 ^{ab}	65.4 ^a	58.7 ^{ab}	54.6 ^a	64.9 ^a	54.1 ^b	63.5	55.6	69.1	49.4	NS	P < 0.06	P < 0.01	4.13	
flavour ⁶	52.0	50.4	49.7	52.5	51.1	50.6	51.1	50.6	49.5	54.5	NS	NS	NS	2.34	
juiciness ⁶	50.6	40.2	47.0	45.7	44.5	47.3	44.1 ^b	44.8	45.6 ^b	55.6 ^a	P < 0.05	NS	NS	3.66	
acceptability ⁶	46.7	43.2	45.8	50.0	42.4 ^a	50.8 ^b	43.7 ^{ab}	49.0 ^{bc}	38.4	55.0 ^c	P < 0.05	NS	P < 0.01	3.82	

¹CO₂ = 90% Carbon dioxide in air, exposure time of 1.8 minutes; Elect = Head to heart electrical stunning, 1.3 amps for 4 seconds

²S = low voltage electrical stimulation for 15 seconds, 4 minutes post slaughter using rectal probe and stick wound clip, L = low voltage electrical stimulation for 60 seconds, 4 minutes post slaughter using rectal probe and stick wound clip, none = no stimulation.

³Chilling = sides chilled at either 2 or 14°C until a pH < 5.8 obtained then chilled at 2°C.

⁴NS = no significant difference, ^{ab} within rows and treatment combinations, means with different superscripts differ significantly (P<0.05), ⁵ 1 = very tender and 100 = very tough,

⁶ 1 = undesirable flavour, extremely dry, unacceptable and 100 = desirable flavour, juicy and acceptable

Table 5.9 Meat quality at rigor and 4 days post slaughter and pH at 3 hours post slaughter in experiment 8 for pork *M. longissimus thoracis et lumborum* after carbon dioxide or electrical head to heart stunning, low voltage electrical stimulation for 15 seconds, 60 seconds or no low voltage electrical stimulation and chilling at 2 or 14°C.

	Stunning ¹ x Stimulation ²				Chilling ³		Significance ⁴	
	Elect	CO ₂ - None	CO ₂ - S	CO ₂ - L	2°C	14°C	Stunning x Stimulation	Chilling
pH 3 hours	6.24 ^a	6.27 ^a	5.83 ^b	5.59 ^c	6.03 ^a	5.94 ^b	P < 0.001	P < 0.01
Rigor								
pH	5.73 ^{ab}	5.89 ^b	5.65 ^a	5.56 ^a	5.73	5.69	P < 0.05	NS
Temperature (°C)	13.2 ^a	13.1 ^a	14.1 ^{ab}	14.8 ^b	12.1 ^a	15.2 ^b	P < 0.05	P < 0.001
Drip loss (%)	2.0 ^a	2.6 ^{ab}	4.4 ^{bc}	5.8 ^c	3.9	3.5	P < 0.05	NS
L*	45.8 ^a	45.1 ^a	48.2 ^{ab}	50.2 ^b	46.7	47.9	P < 0.05	NS
a*	5.0 ^{ab}	4.4 ^a	4.7 ^a	5.7 ^b	4.7	5.1	P < 0.05	NS
b*	2.6	2.4	2.8	3.4	2.7	2.9	NS	NS
4 days								
pH	5.55 ^b	5.58 ^b	5.50 ^a	5.51 ^{ab}	5.53	5.53	P < 0.05	NS
L*	48.0	47.0	46.9	50.7	48.7	47.6	NS	NS
a*	5.4 ^a	5.2 ^a	5.1 ^a	6.2 ^b	5.5	5.7	P < 0.01	NS
b*	3.2 ^a	3.2 ^a	3.1 ^a	4.1 ^b	3.2 ^a	3.7 ^b	P < 0.05	P < 0.05

¹CO₂ = 90% Carbon dioxide in air, exposure time of 1.8 minutes; Elect = Head to heart electrical stunning, 1.3 amps for 4 seconds

²S = low voltage electrical stimulation for 15 seconds, 4 minutes post slaughter using rectal probe and stick wound clip, L = low voltage electrical stimulation for 60 seconds, 4 minutes post slaughter using rectal probe and stick wound clip, none = no stimulation.

³Chilling = sides chilled at either 2 or 14°C until a pH < 5.8 obtained then chilled at 2°C.

⁴NS = no significant difference, ^{ab} within rows and treatment combinations, means with different superscripts differ significantly (P<0.05),

Table 5.10 Purge at 4 and 8 days post slaughter and cooking loss at rigor, 1, 2, 4, and 8 days post slaughter in experiment 8 for pork *M. longissimus thoracis et lumborum* after carbon dioxide or electrical head to heart stunning, low voltage electrical stimulation for 15 seconds, 60 seconds or no low voltage electrical stimulation and chilling at 2 or 14°C.

	Stunning ¹ x Stimulation ²				Significance ³	
	Elect	CO ₂ - None	CO ₂ - S	CO ₂ - L	Stunning x Stimulation	SED
Cooking loss (%)						
rigor ⁴	31.1 ^b	28.6 ^a	31.9 ^b	32.0 ^b	P < 0.01	1.03
1 day	33.9	33.8	33.9	32.9	NS	1.20
2 days	34.4	34.3	34.0	33.3	NS	1.08
4 days	34.5 ^{ab}	36.8 ^b	33.8 ^a	33.7 ^a	P < 0.05	1.05
8 days	35.2	35.8	34.5	33.7	NS	0.96
Purge (%)						
4 days	2.2 ^a	2.8 ^{ab}	4.8 ^{bc}	6.1 ^c	P < 0.05	1.17
8 days	4.4 ^a	4.4 ^a	6.1 ^{ab}	8.0 ^b	P < 0.05	1.31

¹CO₂ = 90% Carbon dioxide in air, exposure time of 1.8 minutes; Elect = Head to heart electrical stunning, 1.3 amps for 4 seconds

²S = low voltage electrical stimulation for 15 seconds, 4 minutes post slaughter using rectal probe and stick wound clip, L = low voltage electrical stimulation for 60 seconds, 4 minutes post slaughter using rectal probe and stick wound clip, none = no stimulation.

³NS = no significant difference, ^{ab} within rows and treatment combinations, means with different superscripts differ significantly (P<0.05),

⁴rigor = 1 hour post rigor as determined by pH < 5.8

Table 5.11 Myofibrillar fragmentation index and sarcomere length measurements at rigor in experiment 9 for lamb *M. longissimus thoracis et lumborum* after low voltage electrical stimulation for 50 seconds at 4 minutes post slaughter or no electrical stimulation.

	Stimulation ¹		Significance ²	
	None	50 seconds	Stimulation	SED
sarcomere length	1.36 ^a	1.63 ^b	P < 0.01	0.088
MFI ³	35.6	35.5	NS	2.94

¹none = no stimulation, 50 sec = low voltage electrical stimulation 4 minutes post slaughter for 50 seconds using a rectal probe and stick wound clip

²NS = no significant differences, ^{ab} within rows, means with different superscripts differ significantly (P<0.05), No other significant treatment effects or interactions occurred other than those presented in the table

³MFI = myofibrillar fragmentation index

Table 5.12 Sarcoplasmic and myofibrillar protein solubility (mg/g) at 1, 3 and 5 hours post slaughter and rigor in experiment 9 for lamb *M. longissimus thoracis et lumborum* after low voltage electrical stimulation and pelvic or Achilles suspension.

Stimulation ¹		Suspension ²			Significance ³	
None	50 seconds	Achilles	Pelvic	Stimulation	Suspension	SED
Sarcoplasmic protein solubility						
1 hour	52.7	57.8	56.2	54.3	NS	12.25
3 hours	51.7	53.2	50.9	53.9	NS	8.59
5 hours	38.0	38.0	37.0	39.1	NS	7.36
Rigor	43.9	41.1	42.1	42.5	NS	11.28
Myofibrillar protein solubility						
1 hour	107	142	140	108	NS	27.6
3 hours	146	158	141	162	NS	33.2
5 hours	147	139	139	147	NS	17.6
rigor	157	140	147	150	NS	15.54

¹none = no stimulation, 50 seconds = low voltage electrical stimulation 4 minutes post slaughter for 50 seconds using a rectal probe and stick wound clip

²suspension = Achilles tendon or pelvic bone suspension from 20 minutes post slaughter until rigor

³NS = no significant difference,
^{ab} within rows, means with different superscripts differ significantly (P<0.05), No other significant interactions occurred other than those presented in the table

Table 5.13 Warner-Bratzler shear force (WBSF, kg) at rigor, 1, 2, 4, 6, 8, and 10 days post slaughter and percentage change in WBSF in experiment 9 for lamb *M. longissimus thoracis et lumborum* after low voltage electrical stimulation for 50 seconds at 4 minutes post slaughter or no electrical stimulation and pelvic or Achilles suspension.

	Stimulation ¹		Suspension ²		Significance ³		
	None	50 seconds	Achilles	Pelvic	Stimulation	Suspension	SED
rigor ⁴	8.99 ^a	7.52 ^b	8.49	8.03	P < 0.05	NS	0.576
1 day	10.51 ^a	8.78 ^b	10.07 ^a	9.22 ^b	P < 0.05	P < 0.05	0.756
2 days	9.52 ^a	7.57 ^b	8.77	8.31	P < 0.05	NS	0.723
4 days	8.85 ^a	6.62 ^b	8.72 ^a	6.75 ^b	P < 0.05	P < 0.001	0.827
6 days	7.99 ^a	6.55 ^b	7.68 ^a	6.86 ^b	P < 0.05	P < 0.1	0.700
8 days	8.61 ^a	5.77 ^b	7.52 ^a	6.86 ^b	P < 0.01	P < 0.1	0.794
10 days	7.78 ^a	5.22 ^b	7.25 ^a	5.75 ^b	P < .001	P < 0.01	0.614
% change WBSF ⁵	4.8	1.6	-6.5 ^a	13.0 ^b	NS	P < 0.05	7.75
rate ⁶	0.36	0.97	0.07	1.26	NS	P = 0.08	0.710

¹none = no stimulation, 50 seconds = low voltage electrical stimulation 4 minutes post slaughter for 50 seconds using a rectal probe and stick wound clip

²suspension = Achilles tendon or pelvic bone suspension from 20 minutes post slaughter until rigor

³NS = no significant difference, ^{ab} within rows, means with different superscripts differ significantly (P<0.05), No significant interactions occurred other than those presented in the table

⁴rigor = 1 hour post rigor as determined by a pH < 5.8

⁵% change WBSF = percentage change in Warner-Bratzler peak shear force from rigor to 4 days post slaughter

⁶rate = rate of ageing from rigor to 10 days post slaughter as determined by fitting a exponential decay equation

Table 5.14 Meat quality at rigor and 4 days post slaughter and pH at 3 hours post slaughter in experiment 9 for lamb *M. longissimus thoracis et lumborum* after low voltage electrical stimulation for 50 seconds at 4 minutes post slaughter or no electrical stimulation and pelvic or Achilles suspension.

	Stimulation ¹				suspension ²				stimulation x suspension				Significance ³			
	none	50 sec	ach	pel	none ach	none pel	stim ach	stim pel	stimulation	suspension	stim*susp	SED				
pH 3hr	6.04 ^a	6.42 ^b	6.23	6.24	6.40	6.44	6.05	6.04	P < 0.001	NS	NS	0.076				
Rigor																
rate ⁴	0.11	0.29	0.16	0.23	0.12	0.10	0.20	0.37	NS	NS	NS	0.149				
pH	5.91	5.87	5.89	8.87	5.90	5.91	5.87	5.86	NS	NS	NS	0.045				
L*	30.5	31.8	31.2	31.1	30.5	30.6	31.9	31.7	NS	NS	NS	0.81				
a*	15.5 ^a	16.7 ^b	16.1	16.1	15.1 ^a	15.9 ^{ab}	17.1 ^c	16.3 ^{bc}	P < 0.01	NS	P < 0.01	0.41				
b*	5.6 ^a	6.2 ^b	5.9	5.9	5.4 ^a	5.9 ^{ab}	6.5 ^b	5.8 ^a	P < 0.05	NS	P < 0.01	0.31				
4 days																
pH	5.56	5.60	5.59	5.57	5.57	5.54	5.56	5.60	NS	NS	NS	0.056				
L*	29.9 ^a	31.6 ^b	30.6	31.0	29.5	30.2	31.7	31.8	P < 0.05	NS	NS	0.54				
a*	18.0 ^a	19.5 ^b	18.3 ^a	19.1 ^b	17.7	18.5	19.5	19.8	P < 0.001	P < 0.05	NS	0.37				
b*	7.4 ^a	8.4 ^b	7.7 ^a	8.2 ^b	7.2	7.8	8.7	8.6	P < 0.01	P < 0.05	NS	0.33				

¹none = no stimulation, 50 seconds = low voltage electrical stimulation 4 minutes post slaughter for 50 seconds using a rectal probe and stick wound clip

²ach = Achilles tendon suspension, pel = pelvic bone suspension from 20 minutes post slaughter until rigor

³stim*susp = stimulation x suspension,

^{ab} within rows, means with different superscripts differ significantly (P<0.05), NS = no significant differences

⁴rate = rate of pH decline from 30 minutes post slaughter to rigor as determined by pH < 5.8

Table 5.15 Purge at 4 and 10 days post slaughter and cooking loss at rigor, 1, 2, 4, 6, 8 and 10 days post slaughter in experiment 9 for lamb *M. longissimus thoracis et lumborum* after low voltage electrical stimulation for 50 seconds at 4 minutes post slaughter or no electrical stimulation and pelvic or Achilles suspension.

Purge (%)	stimulation ¹		suspension ²				stimulation x suspension				Significance		
	none	50 sec	ach	pelvic	none ach	none pelv	stim ach	stim pel	Stimulation	Suspension	stim x susp	SED	
4 days	5.5	4.2	5.2	4.6	5.9	5.1	4.4	4.1	NS	NS	NS		1.67
10 days	7.3	7.6	7.1	7.8	7.7	6.9	6.5	8.7	NS	NS	NS		1.45
Cooking loss (%)													
rigor	32.2	31.6	31.5	32.3	32.2	32.3	30.9	32.4	NS	NS	NS		1.41
1 day	34.7	33.2	34.2	33.8	35.1	34.3	33.3	33.2	NS	NS	NS		1.05
2 days	34.7	34.3	34.4	34.6	34.5	34.8	34.3	34.4	NS	NS	NS		1.30
4 days	36.3	35.1	36.2 ^a	35.1 ^b	36.7	35.9	35.8	34.3	NS	P < 0.05	NS		1.07
6 days	35.8	35.4	35.5	35.7	35.7	35.9	35.3	35.5	NS	NS	NS		0.85
8 days	36.8 ^a	34.9 ^b	35.7	36.0	36.9	36.7	34.4	35.4	P < 0.05	NS	NS		0.96
10 days	35.6	34.4	35.4 ^a	34.6 ^b	35.7 ^b	35.6 ^{ab}	35.2 ^{ab}	33.6 ^a	NS	P < 0.05	P < 0.05		1.13

¹none = no stimulation, 50 seconds = low voltage electrical stimulation 4 minutes post slaughter for 50 seconds using a rectal probe and stick wound clip

²ach = Achilles tendon suspension, pel = pelvic bone suspension from 20 minutes post slaughter until rigor

³stim*susp = stimulation x suspension,

^{ab} within rows, means with different superscripts differ significantly (P<0.05), NS = no significant differences

Table 5.16 Effects of rate of pH decline grouping on meat quality and tenderness of pork *M. longissimus thoracis et lumborum* from experiments 7 and 8.

Rate of pH decline ¹	Slow	Medium	Fast	Significance	SED
pH rate ²	-0.07 ^a	0.22 ^b	1.22 ^c	P < 0.001	0.126
pH 3 hours	6.15 ^a	6.08 ^a	5.72 ^b	P < 0.001	0.074
time to rigor	7.79 ^a	8.23 ^a	4.41 ^b	P < 0.001	0.649
WBSF ³ rate ⁴	0.97	0.57	0.33	NS	0.484
WBSF rigor ⁵	7.06 ^a	6.92 ^a	6.01 ^b	P < 0.05	0.362
WBSF 1 day	7.83 ^a	7.11 ^a	5.79 ^b	P < 0.01	0.567
WBSF 2 days	7.18 ^a	6.27 ^{ab}	5.42 ^b	P < 0.01	0.583
WBSF 4 days	6.97 ^a	6.31 ^a	4.83 ^b	P < 0.001	0.545
WBSF 8 days	6.48 ^a	5.36 ^b	4.78 ^b	P < 0.01	0.478
s. protein sol. ⁶	48.5	38.7	47.4	NS	5.25
pH rigor	5.83 ^a	5.78 ^a	5.60 ^b	P < 0.001	0.051
Drip loss (%)	3.18 ^a	2.83 ^a	5.07 ^b	P < 0.001	0.589
L* rigor	44.03 ^a	45.79 ^a	49.69 ^b	P < 0.001	1.008
pH 4 days	5.50 ^a	5.48 ^a	5.40 ^b	P < 0.01	0.026
L* 4 days	47.95 ^a	48.53 ^a	52.06 ^b	P < 0.001	0.688
S.L. ⁷ rigor	1.64 ^a	1.78 ^a	1.91 ^b	P < 0.001	0.058
S.L. 4 days	1.74 ^a	1.85 ^b	1.92 ^b	P < 0.01	0.053
MFI ⁸ rigor	43.1	45.3	46.2	NS	5.57
MFI 4 days	72.2 ^{ab}	88.1 ^b	68.1 ^a	P < 0.05	8.40

¹Muscles grouped according to the rate constants for pH decline - slow $k < 0.10$, medium $0.11 < k < 0.40$, fast $k > 0.41$

²pH rate = rate of pH decline from slaughter to rigor as determined by fitting an exponential decay equation,

³WBSF = Warner Bratzler peak shear force (kg)

⁴WBSF rate = rate of ageing from rigor to 8 days post slaughter as determined by fitting an exponential decay equation,

⁵rigor = 1 hour post rigor as determined by pH < 5.8,

⁶s.protein sol. = sarcoplasmic protein solubility at rigor (mg/g)

⁷S.L. = sarcomere length (μm),

⁸MFI = myofibrillar fragmentation index

^{abc} within rows, means with different superscripts differ significantly ($P < 0.05$), NS = no significant differences

Table 5.17 Effects of rate of pH decline grouping on meat quality and tenderness on lamb *M. longissimus thoracis et lumborum* from experiment 9.

Rate of pH decline ¹	slow	medium	Significance	SED
pH rate ²	0.01 ^a	0.38 ^b	P < 0.001	0.045
pH 3 hours	6.36 ^a	6.05 ^b	P < 0.001	0.064
time to rigor	9.41 ^a	8.00 ^b	P < 0.05	0.632
WBSF ³ rate	0.27	1.39	NS	0.712
WBSF rigor ⁴	8.54	8.11	NS	0.475
WBSF 1 day	9.81	9.57	NS	0.649
WBSF 2 days	9.20 ^a	7.62 ^b	P < 0.05	0.702
WBSF 4 days	8.08	7.15	NS	0.695
WBSF 6 days	7.42	6.87	NS	0.598
WBSF 8 days	8.11 ^a	5.59 ^b	P < 0.01	0.603
WBSF 10 days	6.81 ^a	5.48 ^b	P < 0.05	0.609
sarc protein ⁵ rigor	44.48 ^a	39.99 ^b	P < 0.01	1.788
my protein ⁶ rigor	154.9	140.7	NS	14.79
pH rigor	5.91	5.87	NS	0.0319
pH 4 days	5.55	5.60	NS	0.034
L* rigor	31.36	30.88	NS	0.598
a* rigor	15.89	16.43	NS	0.349
L* 4 days	30.68	30.71	NS	0.541
a* 4 days	18.72	18.99	NS	0.383
SL ⁷ rigor	1.47	1.53	NS	0.080
MFI ⁸ rigor	35.3	36.0	NS	2.81

¹muscles grouped according to rate constants for pH decline slow $k < 0.20$, medium $0.21 < k < 0.74$

²pH rate = rate of pH decline of LTL muscle as determined by fitting an exponential decay equation,

³WBSF = Warner-Bratzler peak shear force (kg)

⁴rigor = 1 hour post rigor as determined by pH < 5.8,

⁵sarc.protein = sarcoplasmic protein solubility (mg/g),

⁶my protein = myofibrillar protein solubility (mg/g)

⁷SL = sarcomere length (μm)

⁸MFI = Myofibrillar fragmentation index,

^{ab} within rows, means with different superscripts differ significantly ($P < 0.05$), NS = no significant differences

6. GENERAL CONCLUSIONS:

Tenderness is one of the most important attributes of meat to the consumer. To ensure continual retail sales in a highly competitive meat market, pork tenderness must be consistently supplied while maintaining economical production methods. Furthermore, there is no use in producing tender meat without ensuring good meat colour, water holding capacity and flavour. One such production method reported to have many economical advantages over conventional boning is accelerated boning which involves the removal of the muscles from the carcass prerigor. However accelerated boning has been reported to reduce tenderness but may improve other pork quality traits particularly when PSE conditions occur.

There are many factors that can contribute to pork tenderness and overall quality including breed, pre-slaughter handling, stunning, slaughter floor procedures, boning methods, chilling conditions, post rigor handling and ageing of the meat. Probably the most widely reported factor influencing tenderness is cold toughening. However, the majority of cold toughening work has been conducted on beef and lamb due to the greater susceptibility of these species to cold toughening. Cold toughening can still occur in pork thus a need existed for investigation of the impact of cold toughening on pork tenderness. Thus alternate boning methods to conventional boning were investigated to determine efficient production methods to maximise tenderness and other quality traits.

Most research on tenderness takes samples for initial tenderness values at 24 hours post slaughter. This assumes no ageing has occurred between rigor and 24 hours. An important differentiation between this work and others is the definition of initial tenderness at rigor not 24 hours.

Initially two experiments were conducted to determine if sampling location along the LTL would influence the Warner-Bratzler shear force (WBSF) results and to determine the rate of ageing of Australian pork using standard genetics, transport, handling, lairage, stunning and slaughter conditions employed throughout this thesis. From these experiments it was concluded that the location along the LTL should not influence the WBSF values but cooking loss values will increase from the cranial to

the caudal end of the muscle. Therefore care was employed during randomisation of samples to take this effect on cooking loss into consideration. The rate of ageing for pork following rigor boning was determined by fitting an exponential decay equation, which identified that 80% of ageing occurred by 4 days post slaughter.

Cold toughening is greatly influenced by the time of carcass boning following slaughter, in particular accelerated boning. Upon examination, accelerated boning combined with 0°C chilling did reduce tenderness of pork *longissimus* muscle. This reduction in tenderness could be attributed to both cold shortening and a reduction in proteolysis. The reduction in proteolysis can be attributed to the lower temperatures early post slaughter slowing the onset of proteolytic activity and due to cold shortening preventing the enzymes from degrading the myofibrils. Furthermore, this cold shortening also resulted in alterations to other meat quality attributes, increasing drip loss and decreasing surface lightness. Therefore further work was carried out to investigate effective ways to overcome the toughness problems associated with accelerated boning.

One method previously reported to improve tenderness following accelerated boning is temperature conditioning. However conflicting findings on the ideal temperature to optimise tenderness have been reported. Furthermore, the length of time the muscles are conditioned at these temperatures is variable. The study conducted here found temperature conditioning at 14°C until rigor was an effective way to improve pork tenderness and reduce drip loss following accelerated boning. Temperature conditioning at 14°C prevented the cold toughening observed with chilling at 0°C, the reduced proteolysis observed with chilling at 7°C and increased protein denaturation observed with chilling at 21°C. It was postulated that the increased protein denaturation seen following chilling at 21°C resulted in reduced improvements in tenderness due to possible denaturation of the tenderising enzymes, a finding not previously reported in pork. Furthermore, the protein denaturation following chilling at 21°C resulted in a higher L* and an increased drip loss. The rate of tenderisation of pork chilled at 0, 7, 14 and 21°C following accelerated boning has also not been reported in pork previously with maximum improvements in tenderness occurring with chilling at 14°C. Thus chilling at 14°C is the recommended temperature to optimise tenderness, tenderisation and meat quality as originally hypothesised. However, post slaughter storage is required to

maximise the benefits of chilling at 14°C as the improvements in tenderness were not observed until 4 days post slaughter.

The second method investigated to overcome tenderness problems following accelerated boning was calcium chloride infusion. Calcium chloride was effective in improving tenderness and the time of infusion was not important, disproving the original hypothesis that infusion at 0.5 hours post slaughter would result in greater improvements than infusion at 6 hours post slaughter. However, calcium chloride did not reduce the ageing rate of pork with 80% of the improvements in tenderness occurring at 1 day post slaughter for all treatments. Furthermore the need for temperature conditioning at 14°C following accelerated boning was no longer required when calcium chloride infusion was employed as originally hypothesised. Thus calcium chloride has the benefit of allowing muscles that have undergone accelerated boning to be chilled faster and thereby reducing the risk of microbial growth relative to improving tenderness by temperature conditioning at 14°C. Although tenderness was improved, calcium chloride had a detrimental influence on drip loss and purge, important characteristics influencing initial purchase of pork.

Several mechanisms are believed to be involved in the improvement in tenderness following calcium chloride infusion. Probably the most important factor seen here in pork as a result of calcium chloride infusion and not previously reported was the accelerated rate of pH decline post slaughter which would have resulted in an alteration in ionic strength and altered the protein to protein interactions thereby improving tenderness. Furthermore, calcium chloride may have played some role in increasing muscle contraction as indicated by the shorter 4 day sarcomere length measurements. However the infusion of calcium chloride did not appear to alter proteolytic activity with no differences in myofibrillar fragmentation being detected.

The third method used to investigate the ideal method of improving tenderness following accelerated boning was post slaughter storage. This experiment was also designed to determine the ageing patterns of pork following accelerated boning, similar to those reported in the literature for conventionally boned meat. An improvement in tenderness with post slaughter storage was prevented following accelerated boning and chilling at 0°C as originally hypothesised. However, due to the collection of samples prior to the onset of rigor due to extremely slow rates of

rigor onset and the assumption that DFD conditions may have occurred, the true mechanism preventing improvements in tenderness was not clear. Thus it is postulated that the conditions under which the samples were chilled would have been sufficient to cause cold toughening thereby reducing sarcomere length and reducing proteolytic activity although these effects were not detected during analysis. Thus this cold toughening would have been sufficient to prevent improvements in tenderness following post slaughter storage. However, if rigor is presumed to have occurred by 1.25 days post slaughter, at which time the muscles are at their toughest, ageing does appear to have occurred in muscles chilled at 0°C. A clear exponential decline occurs in muscle tenderness from 1.25 days until 10 days post slaughter, thus indicating that improvements in tenderness are still possible. However the improvements in WBSF are not sufficient to result in tenderness levels that would be acceptable to the consumer. Thus further work is required to determine whether muscles boned pre-rigor and chilled at 0°C do in fact improve in tenderness with post slaughter storage.

However, when pork samples following accelerated boning were chilled at 14°C, 80% of improvements in tenderness were observed to occur by 4 days post slaughter, a result not previously reported for pork. Similar ageing patterns to those observed in pork following conventional boning occurred following chilling at 14°C due to the prevention of cold shortening and promotion of proteolytic activity as originally hypothesised. Furthermore, the improvement in tenderness with 4 days ageing for muscles following accelerated boning and chilling at 14°C seen in experiments 4, 5 and 6 suggests that this method of boning is potentially more effective at ensuring tenderness than conventional boning.

Conflicting views in the literature exist about the length of time required to maximise tenderness of conventionally boned pork. Furthermore, conflicting results were also observed for the rate of ageing in different experiments following rigor boning when muscles were chilled at 2°C. Fifty percent of ageing occurred within 2 days post slaughter in experiment two while 50% of ageing occurred after 6 days storage in experiment five. This variation in ageing rate was postulated to be due to different rates of pH decline with slower rates of pH decline resulting in a slower rate of ageing. These variations in pH decline were postulated to be induced by different sized pigs between the two experiments, different numbers of pigs in the chiller and/

or climatic variations between the two experiments with chronic cold stress occurring in experiment five.

Conflicting ageing rates were obtained for rigor boned muscle as a consequence of variations in pH decline, thus the influence of pH and temperature decline on the rate of ageing was examined. Electrical stimulation, different stunning methods and temperature conditioning were used to manipulate the pH decline. It is believed that the main influences of pH and temperature decline are due to alterations in sarcomere length, alterations to protein denaturation and alterations to proteolytic activity. When very fast temperature decline occurs with a slow pH decline (temperature < 10°C, pH > 6.0), cold shortening and a reduction in proteolytic activity are likely to occur. At the other extreme, it was postulated that when a slow temperature decline occurs with a corresponding fast rate of pH decline, tenderisation is likely to be prevented due to protein denaturation. It was postulated that the protein denaturation could result in denaturation of the tenderising enzymes themselves and that denaturation of the sarcoplasmic and myofibrillar proteins may alter the level of protein degradation due to changes in the ability of the enzymes to degrade the myofibrillar proteins. Thus an intermediate rate of pH and temperature decline was hypothesised to be optimal in producing tender pork.

In the two experiments designed to investigate the impact of the rate of pH decline on the rate of ageing of pork, absolute WBSF values were improved at all time points post slaughter in both experiments when the rate of pH decline was the fastest. However the rate of ageing was only increased at higher pH decline rates in experiment 7. However, the methods used to alter the rate of pH decline did not result in conditions sufficient to cause protein denaturation as originally hypothesised, thus the impact of protein denaturation on the rate of ageing could not be determined. As postulated, the reduction in tenderness due to a slow rate of pH decline could be attributed to cold shortening as no differences in proteolytic activity were observed and sarcomere length was shorter.

The results from this experiment suggest that carbon dioxide stunning can potentially reduce the rate of pH decline and reduce the rate of ageing, a result not previously reported. The slower rate of pH decline induced by carbon dioxide stunning has the potential to result in cold toughened pork when efficient chilling methods are

employed. Due to the increased use of carbon dioxide stunning in the Australian pork industry, cold toughening has the potential to become a serious problem. The majority of studies previously looking at stunning methods have focused on animal welfare, worker safety, blood splash, colour and drip loss with little attention being focused on tenderness. Thus the findings in this thesis provide a broader knowledge of the influence of carbon dioxide stunning on overall meat quality and tenderness and suggest further work is required to address the possible impact of carbon dioxide stunning on pork eating quality.

In these experiments, it was evident that electrical stimulation was an effective way to improve tenderness of pork. Furthermore, electrical stimulation did not induce PSE, which is a common concern with the use of electrical stimulation in pigs. This lack of PSE was due to extremely rapid rates of pH decline being avoided. However, drip loss was observed to increase following stimulation for 60 seconds and an increase in L* value was observed following stimulation for 15 and 60 seconds. Thus electrical stimulation for 15 seconds could be a viable method of improving pork tenderness and ensuring consumer acceptability. However, the most notable affect on pork quality seen in experiment 7 was the increased drip loss as a result of electrical stunning. The increased drip loss was hypothesised to be due to the increased muscle contraction during stunning resulting in damage to the muscle structure thereby increasing drip loss.

The alteration to the rate of temperature decline in pork resulted in a greater impact on pork tenderness than the alteration to the rate of pH decline which contrasts with previous reports. Chilling at 14°C in experiment 8 reduced WBSF at all times measured and increased the percentage change in WBSF from rigor to 4 days post slaughter but did not alter the rate constant determined for the rate of pH decline. These improvements were attributed to the earlier onset of rigor relative to chilling at 2°C, the prevention of cold shortening as indicated by longer sarcomere length and increased proteolysis as indicated by the increased level of myofibrillar fragmentation.

Finally, the use of electrical stimulation for lamb muscle resulted in similar results to those seen in pork although different muscle fibres occur in the two species. The use of low voltage electrical stimulation resulted in an improvement in tenderness of lamb and can be implemented as a way of preventing cold toughening. Furthermore, the

rate of pH decline appears to be of greater importance to lamb tenderness than pork with improvements in tenderness occurring with a medium rate of pH decline in lamb. Improvements in tenderness due to a medium rate of pH decline did not occur for pork. Due to the greater susceptibility of lamb muscle to cold toughening relative to pork, this improvement in tenderness is of great importance to the lamb industry. However, the treatments were again insufficient to cause extensive protein denaturation, thus the influence of protein denaturation on tenderness could not be determined.

Overall, from the work conducted, it is clear that cold toughening plays a significant role in pork and lamb tenderness, tenderisation and overall meat quality regardless of the boning method employed with cold toughening occurring in muscles following both rigor and accelerated boning. Thus, careful control of boning methods regardless of the boning method is essential to prevent cold toughening. Furthermore, the results presented in this thesis suggest that accelerated boning followed by chilling at 14°C may potentially produce more tender pork than conventional boning methods with increased ageing observed following this treatment in several experiments.

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