THE INFLUENCE OF RAPID pH DECLINE AND HIGH MUSCLE TEMPERATURE ON THE WATER HOLDING CAPACITY AND SOFTNESS OF BEEF

by

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LIST OF ABREVIATION

Word	Abbreviation
Adductor	AD
adenosine triphosphate	ATP
analysis of variance	ANOVA
Biceps femoris	BF
bovine serum albumin	BSA
dark, firm and dry	DFD
dithiothreitol	DTT
electrical stimulation	ES
Ethylene glycol bis (β-aminoethylether) NN-tetraacetic acid	EGTA
Instron compression	IC
Longissimus dorsi	LD
myofibrillar fragmentation index	MFI
pale, soft and exudative	PSE
Psoas major	PM
reddish, firm, normal	RFN
reddish, soft, exudative	RSE
rigor buffer	RB
Semitendenosus	ST
Semimembranosus	SM
sensory evaluation	SE
sodium dodecylsulfate-polyacrylamide gel electrophoresis	SDS-PAGE
Sternomandibularis	STM
temperature	Temp
Warner-Bratzler shear	WBS
water holding capacity	WHC

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THE INFLUENCE OF RAPID pH DECLINE AND HIGH MUSCLE TEMPERATURE ON THE WATER HOLDING CAPACITY AND SOFTNESS OF BEEF

ABSTRACT

The research in this thesis had two aims. The first was to develop an instrumental method to measure the softness of raw meat and the second was to investigate the influence of rapid pH decline and high muscle temperature post-mortem on the water holding capacity (WHC, that is, the muscles' ability to retain fluid) and softness of beef.

For the instrumental analysis of muscle softness, different Instron compression (IC) methods were compared to sensory evaluation (SE) of meat. The instrumental methods involved compression of small muscle blocks (approximate size 4x5x6cm) parallel and perpendicular to the muscle fibre direction at 20 mm depths and also attaching cylindrical cores of meat (4.5 cm diameter) to the compression head to prevent the samples from collapsing. The most suitable method was compression of muscle blocks perpendicular to the muscle fibre direction to 5 mm depth, as this method was able to show that rapid pH decline and high muscle temperature gave softer meat than slow pH decline at a lower temperature. However, this method did not correlate with the SE scores; the SE method was not able to differentiate the variation in softness between samples.

It was hypothesised that rapid pH decline and high muscle temperature post-mortem would lead to increased protein denaturation, reduced WHC, accelerated meat tenderisation and shortening of sarcomeres and hence soft and exudative meat. It was also hypothesised that these effects would be dependent on the muscle fibre type. In one of the experiments, electrically stimulated (ES) beef *Longissimus dorsi* (LD) muscles were hot boned from the carcasses and incubated in 37 and 14 °C water baths until rigor. We found that the pH decline rate of the muscles subjected to the high temperature was almost double that of the medium temperature treatment with increased protein denaturation, softer muscles and lower WHC. There were no differences in the sarcomere length or tenderness on day 1 or day 3 due to the treatments to indicate accelerated tenderisation. Thus the hypotheses that rapid pH decline and high muscle temperature post-mortem would lead to accelerated tenderisation or sarcomere shortening and subsequently exudative meat were not supported.

In another experiment, two chill rates (fast and slow) and two ES times (10 and 40 secs) were used on beef carcasses to obtain a wide range of pH and muscle temperature decline rates. The LD and *Semimembranosus* (SM) muscles were used to study the effects in two different muscles. The differences in pH and temperature decline rates between the four treatments were much higher in the LD muscle than the SM muscle. Although the LD had larger differences in temperature and pH decline rates between treatments than the SM muscle, it had lower protein denaturation levels and higher WHC than the SM. This could have been due to the higher proportion of fast-glycolytic fibre types in the SM than the LD muscle.

It was found that the muscles with higher temperature and faster pH decline tended to be more tender on day 1 than the muscles with lower temperature and slower pH decline and tended to correlate with softer muscles. This supported the hypothesis that higher muscle temperature and faster pH decline tended to cause softer muscles. However, the SM was firmer than the LD muscle and this was perhaps because the shape and structure of the SM muscle affected the softness more than the biochemical effects of rapid pH decline and high temperature.

Overall, it was found that fast pH decline rate and high temperature resulted in greater protein denaturation, reduced WHC, some tendency to show faster tenderisation and hence softer beef muscles. The results of the experiments showed that the WHC has a direct influence on muscle softness.

CHAPTER 1

1.0 LITERATURE REVIEW

1.1 INTRODUCTION

The WHC of muscle is important to the meat industry because weight loss affects financial value as meat is sold by weight and the expelled juice produces a pool of liquid which is unattractive to the consumers. It is also important in processed meat with respect to the water binding capacity of the muscle and the requirement to optimise yield. Many physical properties of meat such as colour, flavour, texture, softness of raw meat, juiciness and tenderness of cooked meat are influenced by the WHC. Good quality meat is juicier with higher palatability (tenderness) and WHC and acceptability than poor quality meat (Warner, 1994).

1.1.1 Meat Softness

Meat which has a high proportion of bound water is firm and has a tight structure that has a dry or sticky feature. This is a quality defect in which a muscle has a high ultimate pH (usually pH>6.0, but the cut-off can be as low as 5.8 and as high as 6.3, with variations existing between countries; Tarrant, 1981) and is referred to as Dark, Firm and Dry (DFD) or dark-cutting (DC). In contrast, meat with poor WHC known as Pale, Soft and Exudative (PSE) is lighter in colour, appears more watery and has a loose structure (Judge *et al.*, 1989; Lawrie, 1991). PSE meat is caused by both rapid pH decline and the attainment of low pH while the muscle temperature is high. PSE is a major problem in pork but under certain cases beef also exhibit conditions similar to PSE with lighter colour and watery characteristics (Sayre and Briskey, 1963; Penny, 1969; Offer, 1991). Fischer and Hamm (1980) found in beef that 64% of the *Psoas major* (PM) muscles that had fast pH fall post-slaughter were similar to PSE pork but less pronounced.

Although it is not formerly reported, soft-watery beef is recognised by the beef industry as a significant problem, particularly in the export market where beef is shipped from Australia to Japan (Graham Trout, personal communication). The first sign of the problem is that there is usually substantially more fluid expelled from the meat and this accumulates in the vacuum bag prior to and during export. Secondly, the Japanese commonly cut their beef into thin slices for stir-fries or for fresh consumption. When the meat is soft and watery, it is not only difficult to cut

into thin slices but is also seen as an inferior quality due to the loss of fluid and its paler appearance. This usually results in complaints. Since the Japanese pay a premium for their beef and their market is one of our major export markets, it is vital that Australian beef is trusted and recognised as of high quality (Graham Trout, personal communication) by either minimising the soft and watery problem or being able to detect the cuts that exhibit the problem so they can be excluded. In addition, soft meat is a problem on the shelves of domestic supermarkets as soft texture looks inferior and is judged by the consumer as a less acceptable product.

Much research has shown that protein denaturation and the structural changes after rigor mortis are two mechanisms which have major effects on the WHC of meat (Sayre and Briskey, 1963; Penny, 1969; Honikel *et al.*, 1981; Monin and Laborde, 1985; Offer and Cousins, 1992; Wilson III and van Laack, 1999; Joo *et al.*, 1999). However, proteolysis and membrane integrity are also thought to play a role in determining the WHC of meat (Honikel *et al.*, 1981; Boakye and Mittal, 1993; Cheah *et al.*, 1995; Lee *et al.*, 2000; Tornberg *et al.*, 2000), although the mechanisms of the effect are unclear. This review will discuss how protein denaturation, the structural changes due to the onset of rigor mortis, muscle proteolysis and membrane integrity can affect the WHC and softness of beef. In addition, methods for measuring proteolysis and meat softness will be reviewed.

1.1.2 The Water Holding Capacity of Meat

Water holding capacity is defined as the ability of meat to retain water and can be expressed by a number of measurements that include water binding capacity, expressible juice, and exudate or drip loss. Loss of water from the muscle occurs when muscle surfaces are cut and exposed and as the carcasses are further cut into smaller pieces for retail where the water loss can exceed 10% (van Lack and Solomon, 1994). The muscle of a freshly killed animal contains about 75% water (Lawrie, 1961), of which 85% is located within the muscle cell, mainly in the vicinity of the thick and thin filaments. The other 15% is located in extracellular spaces. Water in muscle can be classified as "free water" (95%) and "chemically bound" (5%) (Trout, 1988; Lawrie, 1991). The chemically bound water is held tightly by electrostatic interaction with the polar and charged groups of muscle proteins (Judge *et al.*, 1989). The "free water" is held within the muscle by capillary forces mainly between the thick and thin filaments (Offer and Trinick, 1983; Trout, 1988; Lawrie, 1991).

Clearly any changes in the WHC of muscle would come from the "free" water that is free to migrate throughout the muscle structure. Since the major portion of water is held by the myofilaments, changes in this structure such as swelling or shrinkage will affect the water distribution within the muscle. According to Offer and Trinick (1983), the interfilament spacing is affected by pH, sarcomere length, ionic strength, osmotic pressure and whether the muscle is relaxed or in rigor. By varying these factors, the thick and thin filament spacing can vary from 320 to 570A, which corresponds to a threefold change in volume.

1.2 MUSCLE STRUCTURE

There are three types of muscle; cardiac, smooth and skeletal. Cardiac muscle is present in the walls of the heart and smooth muscle occurs in the digestive tract and blood vessels. The skeletal muscle as the name suggests is attached to bones, often through the tendons. Both the skeletal and cardiac muscles are striated due to their alternating light and dark bands as observed under a light microscope (Bechtel, 1986). In general, 35-65% of the carcass weight of meat animals is composed of skeletal muscle (Judge *et al.*, 1989) and is economically important. It is this type of muscle that will be discussed exclusively but briefly in this review.

The structure of muscle is organised in a hierarchy manner with each level being composed of smaller sub-structures (Figure 1.1). Starting from the outside, the muscle is composed of muscle bundles and within these bundles are many smaller sub-units called muscle fibres or muscle cells. Sheaths of connective tissues called the epimysium, perimysium and endomysium respectively separate the muscles, muscle bundles and the muscle fibres. The muscle fibres contain important cell organelles such as the mitochondria, cell nuclei, sarcoplasmic reticulum, myofilaments, T-tubules and energy sources such as glycogen. The major portion of the muscle fibre is the myofilaments which are the proteins responsible for muscle contraction that give rise to movement (Bechtel, 1986; Judge *et al.*, 1989; Lawrie, 1991).

1.2.1 Muscle Fibres

Approximately 80% of the muscle fibre volume is composed of myofibrils (Figure 1.1.1D) which are rod-like structures of approximately 1 μm in diameter (Bechtel, 1986). These fibres sometimes run parallel to the long axis of the muscle, but in some muscles such as the LD, the

fibres run at an angle to the long axis. The fibre lengths vary from a few millimeters up to 34 cm (Bailey, 1972).

The myofibrils consist of alternating light and dark bands (thus causing the visible striations) which gives rise to periodicity known as the sarcomere (Figure 1.1.1E). A sarcomere is a complete unit that is defined from one Z-disk to the next, and contains contractile units called myofilaments which are predominantly the thick and thin filaments (Figure 1.1.1K & 1.1L). The thick filaments consist of the myosin molecules which are described as rod-like molecules with a large spherical head propped up at one end (Judge *et al.*, 1989). Myosin constitutes about 43% of the total muscle proteins and each myosin is comprised of two subunits, the myosin light and heavy chains. The thin filament constitutes 32% of the total muscle proteins and is composed of three proteins (actin, tropomyosin and troponin) of different shapes chemically bound together (Maruyama, 1985). Actin is the major component of the thin filament. The thin filaments are attached to the Z-disk protein, lying in between are the thick filaments that are held together by other proteins, some of which are located in the M-line (Judge *et al.*, 1989).

The arrangement of the thick and thin filaments is a three dimensional lattice. Six thin filaments surround each thick filament and three thick filaments surround each thin filament (Figure 1.1.1F-I). In each muscle fibre there are about 2000 myofibrils of 1 μm diameter (Bailey, 1972). This arrangement becomes very important when various postmortem changes occur that cause the structure to expand or contract, affecting WHC and tenderness.

1.2.2 Myofibrillar Proteins

Apart from proteins of the thick and thin filaments, other myofibrillar proteins that are of importance and significant abundance are α-actinin, connectin (titin), desmin, nebulin, N_2 line proteins and M-line proteins (Figure 1.2). α-Actinin is the main component of the Z-disk and composes about 2% of the total myofibrillar protein. Connectin extends longitudinally along the thick filament from each end of the Z-disk and is about 10% of myofibrillar protein. Some portion of it is elastic which is believed to act as a shock absorber and scaffold for the alignment and structural integrity within the sarcomere (Maruyama, 1985; Bechtel, 1986; Judge *et al.*, 1989).

Desmin constitutes about 1% of myofibrillar protein and is located at the end of the myofibrils. It encircles the Z disks, connects the adjacent myofibrils and plays an important role in structural organisation. Nebulin is a large protein that runs along the thin filament and constitutes about 3% of myofibrillar protein (Maruyama, 1985). The N_2 line is a structure located at the I band that runs parallel to the Z-disks (Maruyama, 1985). It consists of various proteins (Figure 1.3) and is suggested to also contain titin and nebulin (Taylor *et al.*, 1995). M-line proteins constitute about 2% of myofibrillar proteins and act to stabilise the rod portion of the myosin molecules of the thick filament (Judge *et al.*, 1989).

1.2.3 Cell Membrane

The cell membrane is also known as the sarcolemma and it is about 7 nm thick. It consists of carbohydrates, proteins, glycoproteins (mainly on the outer membrane surface) and predominantly phospholipids. It is quite elastic which allows flexibility during stretching and contraction. The cell membrane performs many important functions, some of which are to contain the contractile units and to regulate the uptake and release of molecules by the muscle cell (Bechtel, 1986; Judge *et al.*, 1989).

1.2.4 Cytoskeletal Filaments and Costomeres

The costameres are protein structures that connect the myofibrils to the cell membrane (Figure 1.3). They are located at the surface of the muscle cell and occur periodically along the myofibril at the level of each I-band and exhibit some structural strength (Taylor *et al.*, 1995). Spectrin and vinculin are two major proteins of the costameres. Spectrin links the actin filaments to the membrane proteins and plays an important part in maintaining the shape and elasticity of the cell membrane.

1.3 PROTEIN DENATURATION AND WATER HOLDING CAPACITY

Protein denaturation is any change in native conformation of a protein that leads to one or more changes in the chemical, biological, physical properties of the protein. Denaturation of proteins may affect their structure and charge and hence their binding of water or the way water is held by the muscle (Penny, 1969; Honikel *et al.*, 1981; Offer and Knight, 1988; Offer *et al.*, 1989; van

Lack and Solomon, 1994). The extent of denaturation is indicated by the reduction in protein solubility and extractability relative to the undenatured protein extracts and specifically in myosin by myofibrillar ATPase activity (Penny, 1977; Tarrant and Mothersill, 1977; Wilson and van Laack, 1999; Joo *et al.*, 1999).

1.3.1 The Effect of Temperature and Rapid pH Decline on Protein Denaturation

Rapid pH decline in muscle pre-rigor and/or the attainment of low muscle pH while the temperature is high post-slaughter causes protein denaturation and hence reduced WHC. Tarrant and Mothersill (1977) showed that a greater muscle depth in the carcass resulted in slower temperature decline which accelerated the rate of pH decline and caused increased amount of drip. Many researchers have shown significant correlations between the amount of drip, cooking loss and loss of WHC with rapid pH decline in muscles during the pre-rigor period or with low pH and high temperature (Bendall and Wismer-Pedersen, 1962; Penny, 1977; Babiker and Lawrie, 1983; Klont *et al.*, 1996; Bowker *et al.*, 1999; Geesink *et al.*, 2000; Claeys *et al.*, 2001). As a result, it is essential to understand what and how proteins are denatured. Unfortunately much of the data available is for pork and not for beef.

In the context of WHC, proteins are usually classified into two groups, the sarcoplasmic proteins and the myofibrillar proteins. Although it is perceived that myofibrils hold the bulk of the water in the muscle, denaturation of both groups of proteins has been extensively studied.

1.3.1.1 Sarcoplasmic Proteins

Sarcoplasmic proteins constitute about 30-34% of the total muscle proteins (Lawrie, 1991). They are suspended in the sarcoplasm and play important roles in cellular metabolism. There is a correlation between the concentration of sarcoplasmic proteins and the amount of drip, generally, the lower the drip the higher the protein concentration in the drip and vice-versa (Penny, 1977; Savage *et al.*, 1990).

Savage *et al.* (1990) found in pork that the drip loss ranged from 3.9-15.1% by weight with the protein concentration ranging from 83 to 146 mg/mL in the drip. Penny (1977) reported similar values of 2-12% drip loss with the protein concentration in the drip ranging from 100 to 150

mg/mL. Savage *et al.* (1990) suggested that it is the denaturation and/or precipitation of the sarcoplasmic proteins that causes low protein concentration in a large amount of drip.

Water can be bound to a protein surface, and it is estimated that 0.5 g of hydration water is bound to each gram of muscle proteins. However, since only about 20% of the total meat weight is protein, the binding of water to the surface of protein molecules is too small to account for the observed changes in water content of meat due to drip (Offer and Trinick, 1983). Although there is a correlation between sarcoplasmic protein denaturation and the WHC, there are disputes about whether this is a cause and effect relationship since sarcoplasmic proteins hold only about 3% of the water and hence cannot be responsible for drip loss of more than this (van Lack and Solomon, 1994, den Hertog-Meischke *et al.*, 1997). Since a drip loss of up to 15% in some muscles has been observed, the origin of drip can not be only from the sarcoplasmic proteins but rather is an indication of denaturation of other proteins, possibly causing changes in the muscle structure.

1.3.1.2 Myofibrillar Proteins

Myofibrillar proteins accounts for approximately 50-55% of the total protein content of the muscle (Lawrie, 1991) and their denaturation can be measured by protein solubility and myofibrillar ATPase activity. Sayre and Briskey (1963) reported a loss of myofibrillar protein solubility of pork muscle with rapid pH fall (pH < 6 at 1 hr) post-slaughter regardless of the temperature at rigor onset. Although the ultimate pH was the same at 24 h, samples that had a slower pH decline resulted in less myofibrillar denaturation. With rapid pH decline, the protein solubility was less than 50% at rigor onset and only 25% at 24 h. Furthermore, medium to low pH at the onset of rigor mortis or within the first few hours after slaughter combined with high temperature greatly reduced both the sarcoplasmic and myofibrillar protein solubility.

Using pH at 90 minutes postmortem to measure the rate of pH decline, Penny (1969) found a significant correlation between myosin ATPase activity, protein solubility and WHC. He reported high values of myosin ATPase, protein solubility and WHC between pH 5.9 and 6.9 and decreased values for pH below 5.9. This reveals that the faster the pH decline, as measured by the pH at 90 minutes postmortem, the lower the protein solubility and myosin ATPase activity, which are indicators of greater protein denaturation. Thus the rate of pH decline can be a good indicator of the effects of protein denaturation on WHC.

Penny (1967) used Ca^{2+} ATPase activity, Mg^{2+} ATPase activity and protein solubility with KCl and pyrophosphate solutions to study the effect of pH and temperature on washed rabbit myofibrils. He found that myofibrils were denatured by heating at 35-42 °C at pH values between 4.8-5.6, and that the rate of deactivation of both myosin ATPase activity and loss of protein solubility were similar. Furthermore, the thermodynamic properties such as enthalpies and entropies of activation were similar suggesting that the three properties, Ca^{2+} ATPase activity, Mg^{2+} ATPase activity and protein solubility, were being changed simultaneously. Penny (1967) also found muscles from carcasses which were held at 37 °C for 4 h after rigor resulted in 50% loss of solubility and 30% loss of Ca^{2+} ATPase activity compared with normal post rigor samples.

Correlation coefficients between the WHC of meat and protein solubility have indicated that sarcoplasmic proteins start to lose solubility immediately after death while myofibrillar proteins are more affected later, largely after completion of rigor mortis (Sayre and Briskey, 1963). They said that there is a highly significant negative correlation between myofibrillar protein solubility and high muscle temperature at the onset of rigor mortis. This correlation partly agrees with the work of Offer (1991) and van Laack and Solomon (1994) who suggested that the presence of adenosine triphosphate (ATP) maintains the myosin ATPase activities and hence significantly protects the myosin (myofibrillar protein) from denaturation. However, they (Offer, 1991; van Lack and Solomon, 1994) also reported that the formation of the actomyosin complex between myosin and actin during rigor mortis has even a greater protective effect in preventing the myosin from denaturation.

An important physical change in myofibrillar proteins as a result of denaturation is the shrinkage of the head of the native myosin molecules which appears to explain the reduction in WHC (Offer *et al.*, 1989). It has been found that the heads of the native myosin molecules are 19 nm long, but when treated under conditions similar to those which cause PSE (pH 6.0, 35 °C for 5 or 10 mins) and loss of myosin ATPase activity, the heads shrank to 17 nm. Shorter myosin heads will draw the thick and thin filaments closer thus reducing the interfilament volume and consequently, more water will be expelled into extracellular spaces. This small change is said to be enough to account for the increased myofibrillar shrinkage in PSE meat (Offer *et al.*, 1989; van Lack, 1994) and perhaps helps to explain the excessive loss of WHC in PSE muscle.

1.3.1.3 Sarcoplasmic and Myofibrillar Proteins

So far our discussions on protein denaturation have looked at how sarcoplasmic and myofibrillar proteins denature separately, however, the denaturation of one protein may affect the denaturation of the other. Many authors (Bendall and Wismer-Pedersen, 1962; Penny, 1967; Warner *et al.*, 1997) have reported that denaturation of sarcoplasmic proteins causes some of them to precipitate onto the myofibrils and postulated that this may reduce the WHC of the myofibrils.

However, this may not be a cause and effect relationship. Monin and Laborde (1985) reported that a sarcoplasmic and myofibrillar mixture has better WHC than myofibrils alone and they suspected that it was due to a positive effect of sarcoplasmic protein on the myofibril's WHC. They said that myofibrils are sensitive to pH and incubation temperatures. At high temperature and low pH, this positive interaction is suppressed and the WHC of myofibrils dominate. If sarcoplasmic proteins precipitate onto the myofibrils and reduce their WHC, then they expect the sarcoplasmic and myofibrillar mixture to have lower WHC than myofibrils alone when incubated in conditions denaturing to sarcoplasmic proteins, however, the observed differences were quite small.

Offer (1991) hypothesised that because postmortem glycolysis generally reduces the muscle pH from 7 to about 5.5, some protein denaturation will occur because this low pH is not the physiological pH usually experienced by muscle proteins in the living state. He also suggested, if the extent of glycolysis is abnormally high such that it results in ultimate pH lower than 5.5, then the muscle proteins will suffer more denaturation than usually experienced postmortem. In contrast, DFD meat has higher ultimate pH (above 6) than both PSE and normal meats (pH about 5.5), yet protein denaturation of DFD muscles does not differ from normal muscles while PSE muscles have the highest protein denaturation (Penny, 1969; Judge *et al.*, 1989; Lawrie, 1991; Warner *et al.*, 1997). DFD meat is firmer and has better WHC than normal meat. It is well known that the ultimate pH affects the WHC of meat through the effects on the myofibrillar lattice (Offer and Trinick, 1983; Offer, 1991; van Lack and Solomon, 1994).

1.3.2 Factors Affecting the Rate of pH Decline and Protein Denaturation

There are a number of factors which influence the rate of pH decline, the major ones being electrical stimulation, muscle temperature, muscle fibre type, species and animal factors which include hormonal state and stress, pre-slaughter and during slaughter (Offer 1991). This review

will only discuss the effects of electrical stimulation, temperature and muscle fibre type on the rate of pH decline.

1.3.2.1 The Effect of Electrical Stimulation on pH Decline

Electrical stimulation is used to achieve rapid rigor to prevent cold shortening and associated toughness (Crystall and Devine, 1991; Bendall, 1980). However, since it accelerates postmortem glycolysis and increases the rate of pH decline, greater losses in WHC and drip have been observed in beef carcasses which have been ES possibly due to protein denaturation (Offer 1991).

Electrical stimulation affects the pH decline rate of different muscles differently. By measuring the temperature of beef muscles at 1, 3 and 24 h post-slaughter and chilling the carcass over night at 2.5 °C, Den Hertog-Meischke *et al.* (1997) found that low voltage ES had greater effect on the pH decline of the SM muscle than the LD. The SM muscle had a much faster pH decline and slower temperature fall than the LD, and reached a lower ultimate pH. However the ultimate pH did not differ between ES and non-stimulated muscles. Electrically stimulated samples had lower sarcoplasmic protein solubility than non-ES samples suggesting that one of the effects of ES is increased sarcoplasmic protein denaturation. The SM had higher drip loss and filter paper wetness than the LD and this was probably caused by the different pH decline rates. Since the ultimate pH and sarcomere lengths did not differ between ES and non-ES in the SM muscles, the drip loss difference may have been due to additional myosin denaturation by high temperature/low pH condition caused by ES. Den Hertog-Meischke *et al.* (1997) accounted for these effects due to the differences in fibre types of the SM and the LD muscles. In addition, the above effects could also be accounted for by temperature differences due to the location and thus depth of the muscles within the carcass.

Babiker and Lawrie (1983) found that ES and incubation of bovine LD muscles at 30 °C resulted in a much faster pH decline than just incubation at 40 °C without electrical stimulation. According to Bowker *et al.* (1999) and Maribo *et al.* (1999), the faster pH decline should have also resulted in higher cooking loss or drip loss, but surprisingly Babiker and Lawrie (1983) found no significant differences in the WHC between the stimulated and non-stimulated muscles. However, they (Babiker and Lawrie, 1983) did find higher loss of WHC in muscles that had been ES and incubated at 40 °C than muscles that were ES but incubated at 30 °C. The authors held the samples at the specified temperatures for 5 h and thereafter the drip and cook loss tests were

carried out. Perhaps this was too short a time to allow drip formation in order to observe significant differences between treatments.

1.3.2.2 The Effect of Temperature on pH Decline

Of all the factors that affect the rate of pH decline, temperature is the most important. Sayre and Briskey (1963) reported in an experiment they conducted that the sarcoplasmic protein solubility of muscles held at temperatures below 35 °C or above 35 °C before the onset of rigor were all similar. However, at onset or just after rigor, muscle temperature affected the solubility of sarcoplasmic proteins if the pH fell below 6.0. Muscles that entered rigor above 35 °C with rapid pH decline (or low pH) gave the lowest protein solubility. Muscles that entered rigor above 35 °C also gave lower sarcoplasmic protein solubility even though their ultimate pH was higher than samples which entered rigor at a lower temperature but had similar or lower ultimate pH. On the other hand, when muscles entered rigor at a pH > 6.0, or had a high ultimate pH then the protein solubility was not affected by temperature, and hence retained high protein solubility. These results emphasise the importance of temperature interaction with the rate of pH decline at rigor onset (Sayre and Briskey, 1963).

Muscle depth also illustrates the effects of temperature on the rate of pH decline. Muscles deeper within the carcass will experience higher temperature for a longer period post-slaughter. Tarrant and Mothersill (1977) found that in bovine *Adductor* (AD), *Semitendinosus* (ST) and *Biceps femoris* (BF) muscles, at 8 cm depth, a high temperature of 30 °C lasted for 3 h while the pH values of less than 6 were obtained. The average pH decline rate of the four muscles between 1 and 4 h postmortem at different depths were 0.05-0.07 units/h at 1.5 cm and 0.22-0.29 at 8 cm. This is considered rapid pH decline for beef muscles and examination of these samples at 2 days postmortem revealed paler, softer and wetter appearance at 8 cm than 1.5 depth. The muscles developed a mild PSE as can often be observed in pork.

The effects of temperature on the pH decline as indicated by varying muscle depth is clearly reflected by the denaturation of sarcoplasmic and myofibrillar proteins (Tarrant and Mothersill, 1977). Denaturation of sarcoplasmic proteins is indicated by almost total loss of creatine kinase activity during rigor onset at 8 cm in the AD and SM muscles and faster depletion of ATP at 8 cm than at 1.5 cm depth. Furthermore, Tarrant and Mothersill (1977) showed that the ATPase

activity at 8 cm was 29% lower than at 1.5 cm depth in beef AD muscle and the myofibrillar solubility was 32% lower at 8 cm than at 1.5 cm.

1.3.2.3 The Effect of Muscle Fibre Type on pH Decline

Muscle fibres can be basically classified into three types, red, white and intermediate depending on their physiological and energy metabolism characteristics. It is rare that muscles are composed of all white, all red or all intermediate fibres and are usually a mixture of both. Hence, red muscles are those with higher portion of red fibres than found in white muscles and vice versa. The predominant fibre often determines the quality of meat (Judge *et al.*, 1989; Lawrie, 1991).

Red and white muscle fibres are slow and fast twitch muscle fibres respectively. Red fibres contract more slowly but can sustain the action for longer periods. This requires constant supply of energy and thus red fibres have higher oxidative metabolism because of greater utilisation of the Krebbs cycle and electron transport chain to generate ATP. As a result of this oxidative process there is a higher demand for oxygen hence red fibres contain more myoglobin, more mitochondria, and have a smaller size. . Red fibres have a higher lipid content which serve as an oxidative fuel whereas white fibres have a higher glycogen content for immediate supply of ATP. The reason for this is that white fibres undergo higher anaerobic glycolysis for production of ATP from the conversion of glycogen to lactic acid that is used for rapid muscle contraction (Judge *et al.*, 1989; Lawrie, 1991).

It is commonly known that pre-slaughter stress depletes glycogen from muscle fibres and the different types of muscle fibres are more or less affected by stress due to the differences in energy metabolism and the initial store of glycogen level. The ultimate pH of red muscles is often higher due to the lower glycogen content and their buffering capacity is lower than that of white muscles. Therefore, the tendency of muscles to develop DC or PSE character will depend on the relative amount of fast and slow twitch fibres they contain. Whereas white muscles will be susceptible to PSE because of their higher glycogen content and faster energy metabolism (Judge *et al.*, 1989; Lawrie, 1991).

In bovine muscles, Tarrant and Mothersill (1977) found that different muscles of the round (AD, SM, ST and BF) had different temperature and pH decline rates due to different fibre types in the muscles and different depths of the muscle from the surface of the carcass. Their results also

implied that due to different fibre types in different muscles, the highest pH decline rate did not result in the highest amount of drip. The ST muscle reached pH 6 in 2.8 h while the SM reached pH 6 in 4.1 h and yet the ST muscle produced 16.7% drip compared to 19.4% drip in the SM. They did not measure the protein denaturation or structural changes and thus could not account for the differences in the WHC measurements.

1.3.2.4 Methods for Measuring Protein Denaturation

Protein Solubility

Protein solubility (sarcoplasmic and myofibrillar) and myofibrillar ATPase activities are some of the methods that can be used to measure protein denaturation. The sarcoplasmic protein solubility measures the amount of proteins that can be solubilised from the sarcoplasm using a weak ionic strength buffer. The total protein solubility measures the total amount of muscle proteins that can be solubilised by a strong ionic strength buffer (Penny, 1969; Wu and Smith, 1987; van Lack and Solomon, 1994; Warner, 1994). The principal idea is that myofibrillar protein solubility increases with the ionic strength of the buffer (Penny, 1969; Wu and Smith, 1987; van Lack and Solomon, 1994; Warner, 1994) and that denaturation of proteins reduces their solubility relative to the undenatured protein (Penny, 1977; Tarrant and Mothersill, 1977). Since the sarcoplasmic proteins are already solubilised in the sarcoplasm, only a weak ionic buffer of similar ionic concentration to the sarcoplasm is required to remove or separate them from undissolved muscle proteins. On the other hand, myofibrillar proteins require a much stronger ionic buffer to solubilise them. Hence, myofibrillar protein solubility is taken as the difference between the total and sarcoplasmic protein solubility (Penny, 1969; Monin and Laborde, 1985; Warner, 1994).

Myofibrillar ATPase Activities

The ATPase methods commonly involve purifying the myofibrils from the whole muscle. The solution is then diluted to a known concentration with the addition of appropriate ions (activator) and ATP. The activity of the enzymes is measured by the amount of inorganic phosphate that is liberated from the break down of ATP by the ATPase enzymes (Penny, 1967; Ouali, 1984; Warner, 1994). If the enzymes are denatured then they will be unable catalyse the reaction and overall their activity will be less compared to the undenatured enzymes. Since these enzymes are located on the myosin head, their denaturation gives an indication of the condition of the myofibrillar proteins.

According to Ouali (1984, 1992), there are three different myofibrillar ATPase activity methods:

1) the Ca-activated myofibrillar ATPase measures the activity and checks the integrity of the myosin molecule in the myofibril in the presence of Ca^{2+} ions (4mM);
2) the Mg-Ca activated myofibrillar ATPase measures the activity of the actomyosin complex in the presence of Mg^{2+} (4mM) and Ca^{2+} (2mM) ions, and
3) the Mg-EGTA modified activity in the presence of Mg^{2+} (4mM) ions and EGTA (1mM, ethylene glycol bis (β-aminoethylether) NN-tetraacetic acid), which is an indicator of the regulatory complexes tropomyosin and troponin.

Ca-activated myofibrillar ATPase activity is the most commonly used of the three methods as an indication of protein denaturation in the myosin head. When the myosin molecules are denatured, the head of the molecules shrink which can subsequently reduce the myofibrillar lattice spacing (Offer and Trinick, 1983; Rao *et al.*, 1989; Guignot *et al.*, 1993).

1.4 MUSCLE STRUCTURAL CHANGES DUE TO ONSET OF RIGOR MORTIS AND THEIR EFFECTS ON WHC

1.4.1 Rigor Mortis

Rigor mortis is the loss of extensibility of muscles after death (Judge *et al.*, 1989). It forms as a result of permanent cross-bridges between actin and myosin that develop once ATP stores are depleted. ATP is normally required to release bonds between actin and myosin. The onset of rigor mortis can be observed when muscles begin to lose elasticity and extensibility and develop shortening and tension. Unrestrained muscles are free to shorten more than restrained muscles. Rigor shortening is different from normal contraction because in normal contraction there is only about 20% binding of the actomyosin, however, in rigor there is binding in nearly all areas of overlap between myosin and actin. The rate of rigor development post-slaughter differs between animals and muscles but is closely related to pH decline and fibre type because both are related to the metabolism of glycogen and ATP. The faster the pH decline the quicker will be the onset of rigor (Sayre and Briskey, 1963; Honikel *et al.*, 1981; Lee *et al.*, 2000; Tornberg *et al.*, 2000).

1.4.2 Muscle Shortening

The degree of shortening that a muscle undergoes as it enters rigor mortis is temperature dependent. Above 10-15 °C, shortening increases with increasing temperature and at a relatively high temperature (37-50 °C) severe heat shortening is likely to occur. Also with the higher temperature range, early onset of rigor mortis may be induced (Judge *et al.*, 1989). However, if the muscle temperature is reduced to less than 10 °C before onset of rigor then cold shortening is also likely to occur. The causes of cold-shortening relate to the release of Ca^{2+} or failure of the calcium pump of the sarcoplasmic reticulum due to the low temperature. During onset of rigor mortis muscles which are held on the carcass have less tendency to shorten, but certain parts of the muscle may have limited freedom to contract and may do so while other parts lengthen to maintain the same overall length (Lawrie, 1991).

Thaw rigor is a severe type of shortening that occurs when muscle that was frozen pre-rigor is thawed. On thawing, the rate of ATP break down will be affected by the rate of pre-rigor freezing; rapidly frozen muscles metabolise ATP faster than slowly frozen muscles and the onset of rigor mortis is also quicker (Lawrie, 1991). The resulting contraction is caused by sudden release of Ca^{2+} into the sarcoplasm and may cause physical shortening of up to 80% of the original length of unrestrained muscles, but more commonly the shortening is 60% (Judge *et al.*, 1989). 30-40% exudation or drip loss can occur if the muscle undergoes thaw rigor (Lawrie, 1991).

Although cold shortening tends to be more severe, both cold and heat shortening will cause sarcomere shortening and hence a reduction in the myofibrillar lattice. In addition, heat shortening can be accompanied by protein denaturation and both of these factors will subsequently lead to loss of WHC of meat and increased drip loss during post rigor storage. The amount of water loss will depend on the severity of the problem (Judge *et al.*, 1989; Lawrie, 1991). Minimum drip occurs if meat or carcasses are stored at 10-15 °C pre-rigor that coincides with minimum shortening. Reducing the muscle temperature from 37 to 15 °C will not only minimise heat rigor but also protein denaturation. However, rapid chilling of pre-rigor muscles may induce cold shortening. Through appropriate chilling and muscle restraint, shortening can be minimised, although in practice, ES of a carcass is far easier to use in a commercial environment.

In beef carcasses, cold shortening does not tend to affect muscles deep in the carcass such as the rump. Due to the high initial muscle temperature and insulation by the carcass, postmortem glycolysis would be completed before refrigeration can lower these muscles below 15°C. Consequently, these muscles are more likely to suffer from the exudative condition similar to PSE pork (Offer, 1991).

1.4.3 Changes in Filament Spacing

Postmortem glycolysis in a typical muscle will normally reduce the initial muscle pH of 7 to an ultimate pH of about 5.5 (Swan, 1993). Associated with the reduction in pH, the electrostatic repulsion between negatively charged myofilaments will decrease. The isoelectric point of muscle proteins where the number of positive and negative charges are equal is at a pH of about 5.0 (van Laack and Solomon, 1994). As the pH approaches the isoelectric point there will be a reduction in the negative groups of the myofibrillar proteins which produces less repulsion. Consequently, the interfilament spacing is reduced and the loss of WHC occurs in addition to the shortening caused by temperature as discussed in section 1.4.2 (Judge *et al.*, 1989).

At pH ranges higher or lower than 5.0, there will be excess negative or positive charge respectively. When this occurs there will be repulsion between filaments resulting in a larger interfilament space which can hold more water. Attachments of the actin filaments to the Z-line, the myosin filaments to the M-line and the actomyosin crosslinks are the constraints to the swelling when the negative charge on the proteins is increased with an increase in pH (Offer and Trinick, 1983).

Furthermore, as a result of rigor and the formation of actomyosin crossbridges, there is a reduction in total volume due to sarcomere shortening as well as fibre diameter shrinkage that leads to smaller filament spacing. The filament spacing decreases to about 4.4% smaller than the original spacing which corresponds to a 9% decrease in cross sectional area and hence volume of the myofibrils (Offer and Trinick, 1983; Offer *et al.*, 1989; Offer and Cousins, 1992; Tornberg *et al.*, 2000). The fluid that is expelled accumulates between the fibre bundles and between fibres. The reduction in volume can be caused not only by rigor development but also by protein denaturation due to the head shrinkage of the myosin molecules and the ultimate pH. Thus, as muscles enter rigor mortis some loss of WHC is inevitable.

If these principles are true then the loss of water due to pH decline is also inevitable. That is, the WHC will be affected by the extent of postmortem pH fall and the lower the ultimate pH, the lower the WHC (Lawrie, 1991).

1.4.4 The Formation of Extracellular Space

There are several theories as to how the cellular water is distributed during the formation of extracellular spaces that lead to drip loss. According to Offer and Trinick (1983), Offer *et al.* (1989) and Offer and Cousins (1992), initially there are no large channels in the *in vivo* muscle and the fibres fill the endomysial network and the fibre bundles fill the perimysial network. At 6 hours postmortem, gaps of variable sizes appear between fibre bundles while by 24 hours, gaps appear between fibres as the myofibrils shrink causing the fibres to shrink. The cellular water accumulates around the perimysial then later around the endomysial network resulting in two extracellular compartments. Gravity then drains these compartments creating the drip.

Honikel *et al.* (1986) suggested that in living muscle, during contraction the muscle cell volume does not change, therefore, the distance between myofilaments within sarcomeres must increase, then decrease during relaxation. During rigor, the fibre diameters decrease which result in a decrease in muscle volume causing the extracellular space to increase, hence water must migrate from intracellular to extracellular space. Decreasing the distance between thin and thick filaments could also cause expulsion of water and influx into the sarcoplasm that will cause dilution of the sarcoplasmic proteins. In shortened sarcomeres this diluting effect is pronounced since the drip is higher.

Penny (1977) found that the amount of extracellular space increases with holding temperature and time post-mortem, up to a limit of about 25%. There is a correlation between the amount of drip and the increase in extracellular space up to a maximum of 25%, thereafter, the extracellular space does not increase even though drip increases due to protein denaturation. During this event, the sarcoplasmic fluid is thought to be transferred to the extracellular space. The rate of increase in extracellular space is different for different muscles.

To express it simply, there is a redistribution of water within the muscle postmortem due to longitudinal shortening of sarcomeres and transverse shrinkage of the fibres. The shrinkage either

leaves the water behind in the extracellular space or pushes the water from the intracellular to the extracellular space that has increased in size due to the cell shrinkage.

1.5 PROTEOLYSIS AND WHC

It is commonly observed that meat becomes more tender during ageing which makes it easier to cut and shear during eating or instrumental analysis. Upon homogenisation, aged meat yields a higher number of small fragments than unaged meat which indicates that the muscle structure is weakened (Davey and Gilbert, 1969; Koohmaraie, 1988). These changes are largely attributed to proteolysis in which certain muscle proteins are degraded hence weakening the structure. There appears to be a significant correlation between tenderisation and the degree of fragmentation of meat, that is, the amount of fragmentation increases with increasing tenderness as muscles age. As a result, the amount of protein degradation occurring in muscles is often correlated with the increase in tenderness: this change in tenderness is an indicator of proteolysis (Olson *et al.*, 1976; Calkins and Davis, 1980; Davis *et al.*, 1980; Koohmaraie, 1992; Ferguson *et al.*, 2000; Lee *et al.*, 2000; Geesink *et al.*, 2001).

If protein degradation occurs to the extent that it disrupts the structural integrity of the muscle fibres, thus changing the myofibrillar lattice structure, then what effects would this have on its water holding capacity? There is extensive research into the relationship between proteolysis and tenderisation, however, the effect of proteolysis on WHC is little known. As the bulk of the water in muscle is held in the myofibrils through capillary forces, it would be expected that the loss of WHC would also relate to the degree of protein degradation and subsequently the change in tenderness. Does this mean there is a relationship between the degree of tenderisation and WHC, or does proteolysis affect only tenderness without much influence on the WHC?

The question is, what is happening to meat during ageing and what proteins are being degraded that results in increasing tenderness? In the following discussion I will review the structural changes due to proteolysis and how these influence the tenderness. Consequently, this may provide better understanding of how the proteins that influence the muscle integrity may also affect the WHC of beef.

Meat is usually refrigerated at 0-2 °C during ageing and under this conditions researchers (Penny, 1980; Ouali, 1990; Ouali, 1992; Dransfield, 1994; Goll *et al.*, 1995; Taylor *et al.*, 1995; Koohmaraie, 1996) have reported the following findings.

1) In beef, after 3 days, breaks at the junction of I-filaments with Z-disk appear, larger gaps appear later with continuous breaks involving many myofibrils. The Z-disk is attached to one end of I-filaments at the fracture site.
2) Degradation occurs in or near the Z-disks, hence weakening the Z-disk.
3) The disappearance of electron density of the M-lines.
4) The loss of transversal alignment of Z-disks and M-lines.
5) Complete loss of Z-structure does not occur until after 10-15 days storage.
6) Muscle can be stretched to about the same length as in the pre-rigor state after a few days ageing at 0-4 °C, but the stretching is not reversible.
7) Stretching breaks I-filaments from their junction with Z-disk.
8) Different fibre types have varying rates of Z disks degradation and the appearance of gaps in I-Z junction also varies between fibre types.
9) In beef, red fibres' Z-disks are twice as thick as white fibres and are claimed to degrade at a slower rate than white. This is also observed in pork and chicken muscles.

1.5.1 Degradation of Z-Disks

There appears to be confusion over the understanding that fragmentation of muscle fibres is due to the Z-disk degradation. The Z-disk is composed mainly of α-actinin, but researchers have not found fragments from this protein within the first 3-4 days at 2°C storage to confirm that the Z-disks are being degraded. It is during this early postmortem period that rapid tenderisation, hence proteolysis occurs (Dransfield, 1994; Uytterhaegen *et al.*, 1994; Alarcon-Rojo and Dransfield, 1995; Taylor *et al.*, 1995; Koohmaraie, 1996). It is only after 14 to 18 days of ageing that the Z-disks lose structural integrity and density during storage at refrigerated temperature and by then much of the tenderisation and major structural changes have already occurred (Ouali, 1990; Koohmaraie, 1992; Taylor *et al.*, 1995). Often it can be seen in electronmicrographs that the Z-disks are still attached to one end of the I-filament when there are breaks in the myofibrils (Penny, 1980; Taylor *et al.*, 1995). There appears to be stronger evidence that the myofibrils do not break in the Z-disks but rather beside the Z-disks, in the I band.

1.5.2 Degradation of Contractile Filaments

The major myofibrillar proteins myosin and actin do not appear to degrade under refrigerated storage. Myofibrillar protein degradation is often detectable by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) immunoblotting using antibodies, but so far there have not been any report showing that the major contractile proteins such as the myosin and the actin are degraded at refrigerated temperature even after weeks of storage.

On the other hand, one of the most significant early findings is the degradation of myofibrillar protein troponin-T and the appearance of smaller polypeptides, well recognised as a 30 kDa component (Penny, 1980). The rate of disappearance of troponin-T (in beef) is over a range of temperatures from 3-35 °C and correlates well with the rate of increase in tenderness (Koohmaraie, 1992). If the understanding that muscle fragmentation occurs due to breaking in the myofibrils, then how do we explain the close correlation between the disappearance of troponin-T and the increase in tenderness, if troponin-T is not part of the Z-disk but is attached to the contractile proteins instead?

The disappearance of troponin-T during ageing of cold shortened muscles does not reduce toughness (Koohmaraie, 1992) and it is also doubtful that the disappearance of troponin-T is related to meat tenderness, apart from the fact that it is a good tenderness indicator (Ouali, 1990). So the question is, if the disappearance of troponin-T is not directly related to tenderisation nor has there been much, if any, evidence to support the Z-disk degradation, what is happening during early postmortem tenderisation?

1.5.3 Degradation of Cytoskeletal Proteins

Cytoskeletal proteins are those that give muscle fibres their structure and integrity (refer to muscle structure in section 1.2). Important proteins such as nebulin, titin and N_2 line proteins (the region where titin and nebulin merge in the I-band), are also degraded during storage of beef (Taylor *et al.*, 1995). Both titin and nebulin extend through the I band where breaks in the myofibrils often occur during post-mortem storage. Titin runs along the whole length of the sarcomere attaching to the myosin filaments and the Z-disks giving the myofibrils their elasticity (Maruyama, 1985). Degradation of these proteins would weaken the connections between the I-band and the Z-disk that consequently weakens the myofibrils.

It is speculated that the increase in tenderness during post-mortem storage is due to the loss of tensile strength and structural integrity of the myofibrils (Ouali, 1990; Koohmaraie, 1992). However, in highly shortened meat, the I-bands are not observed and the A-bands form a continuous band when meat is cooked. Cold shortened meat does not tenderise significantly during storage and it has been suggested that perhaps any change in the thin filaments or Z-disk is masked by the continuous A-bands (Koohmaraie, 1992).

In addition, intermyofibril linkages contain filamin and desmin. Both of these proteins are degraded during early periods of storage which may account for the longitudinal splitting and tearing between the myofibrils as often observed on electron micrographs (Taylor *et al.*, 1995).

1.5.4 Degradation of Costameres

Cell costameres are composed of proteins located at the surface of the muscle cell and contribute to structural strength (Taylor *et al.*, 1995). In questioning the Z-disk theory, Taylor *et al.* (1995) suggested that degradation of the costameres could contribute significantly to the early postmortem tenderisation. They reported that the costameres are almost completely destroyed after 72 h and cause the cell membrane (cell membrane) to separate from the myofibrils. Furthermore, Taylor *et al.* (1995) hypothesised that the costameres are more susceptible to proteolysis from early activation of calpains due to the release of Ca^{2+} close to the surface of the cell. Their degradation may also increase the postmortem osmotic pressure in the muscle cell that further aids tenderisation (Taylor *et al.*, 1995).

Much of meat tenderisation occurs early postmortem and during this period there are breaks within and between the myofibrils (Ouali, 1990; Koohmaraie, 1992). Electronmicrographs often show that the breaks within the myofibrils occur beside the Z-disks and not within the Z-disks during this period. The Z-disks maintain their structural integrity until about 14-18 days storage at refrigerated conditions (Penny 1980, Uytterhaegen *et al.* 1994, Dransfield 1994). The degradation of costameres, intermyofibril linkages (desmin), disruption of the actin filament/Z-disk interaction together with some structural proteins such as filamin and nebulin coincide with the early period of tenderisation, therefore more significance should be given to these proteins rather than the degradation of the Z-disks (Taylor *et al.*, 1995).

1.5.5 Breakdown of the Cell Membrane

In live animals, the cell membrane is essential in maintaining the fluid within the cell (Honikel *et al.*, 1986). However as described above, the costameres which anchor the cell membrane to the myofibrils are degraded by about 72 h postmortem and hence allow the cell membrane to separate from the myofibrils. Boakye and Mittal (1993) found pressed juice increased by 4% from 54.7 on day 0 to 58.5% by day 12. The explanation offered by the authors was that the well ordered structure within and across the myofibrils is broken, the cellular membranes become leaky and intracellular water moves into extracellular fluid. With progressing time, membrane structures disintegrate and water leaves the muscle cell, enhancing pressed juice.

Offer and Cousins (1992) reported that the perimysial-endomysial junction (where the connective tissues perimysium and endomysium merge) is the weakest. When this ruptures, the myofibrils and hence the fibres and fibre bundles are able to shrink. The expelled fluid accumulates in the spaces between the fibre bundles and perimysium. Rupturing of the cell membrane during the shrinkage of the muscle fibre may allow the sarcoplasmic proteins to diffuse into the extracellular space between the fibre and endomysial network. These proteins can cross the endomysial sheets into the space between the fibre bundles and appear as drip. In rigor muscles, there are gaps between fibres and fibre bundles and observation of freshly cut meat surfaces show drip emerges from perimysial gaps rather than within fibre bundles (Offer *et al.*, 1989).

If the above finding of the contribution of the perimysium and in particular, the cell membrane to WHC were accurate, then could we expect these structures to have some effects on the WHC of the muscle during ageing? In particular, if the cell membrane does not maintain its structural integrity during meat ageing, then could there potentially be more drip due to the loss of fluid retention by the cell membrane?

1.5.6 Methods for Measuring Proteolysis

There is no one universally ideal method for measuring proteolysis. Instead several methods are used in combination to provide a supporting confirmation of the properties being measured. These methods commonly include the Warner-Bratzler shear (Warner, 1928; Bouton and Harris, 1978), myofibrillar fragmentation index (MFI) (Davey and Gilbert, 1969; Olson *et al.*, 1976; Culler *et al.*, 1978), sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) and

immunoblotting (Western blotting) of specific proteins (Penny, 1980; Maruyama, 1985). The SDS-PAGE and immunoblotting techniques are qualitative or semi-quantitative and will not be reviewed here, whereas the other methods are quantitative.

1.5.6.1 The Warner-Bratzler shear

There are many instrumental methods used to measure the tenderness of cooked meat, however the WBS is most commonly used in the meat industry and research. Despite the WBS being so commonly used, there are many variations to the method and these include sample size and shape, blade type (i.e. flat or V-shaped), cooking method (boiled or dry cook), cooking temperature and time and the size of the samples being cooked (Bouton and Harris, 1972; Hunt and Hedrick, 1977; Bouton and Harris, 1978; Winger, 1979; Bruce and Ball, 1990; Lepetit and Culioli, 1992; Koohmaraie *et al.*, 1996). Bouton et al (1972) modified the WBS by using a flat blade to shear a rectangular sample of 0.6 x 1.5cm instead of the common 1x1cm rectangular sample sheared by a V-shaped blade. This modification was unique in that the sample is enclosed securely in a cage of the same dimension to prevent it from curling up during shearing and hence minimise variations in the WBS values. The WBS can also be used as a measure of proteolysis if the tenderness of the meat is measured over an ageing period to obtain the rate of change in the tenderness. In meat research, the tenderness can be evaluated by both a taste panel and instrumental method. Some of the advantages of instrumental assessment are that it is cheaper, quicker and does not need a large group of people to obtain some preliminary indication of the tenderness.

1.5.6.2 The Myofibrillar Fragmentation Index

The MFI method is used to measure how readily muscles break into small fragments upon homogenisation (Olson *et al.*, 1976). The theory is that tender meat should break into much smaller fragments than tougher meat. For the same protein concentration, a suspension of more highly fragmented particles should give a higher turbidity, i.e. absorbance, reading than the suspension with less fragmented larger particles. The absorbance values are then multiplied by a fixed factor (e.g. 200) to obtain whole numbers. The higher the MFI values, the greater is the fragmentation and hence the more tender is the meat.

The amount of homogenisation (i.e. homogenisation speed and time) (Davey and Gilbert, 1969; 1976; Culler *et al.*, 1978) and whether the samples are fresh or frozen (Davis *et al.*, 1980; Crouse

and Koohmaraie, 1990) are some of the variations within this method. It is believed that excessive homogenisation would show little differences between tender and tough or aged and unaged meat, while insufficient homogenisation would lead to large variation in the MFI values. In addition some authors (Davey and Gilbert, 1969; Culler *et al.*, 1978; Koohmaraie *et al.*, 1996) do not freeze meat for MFI measurements because they feel that freezing has a negative effect on the muscle structure and hence the consistency of the MFI results. Hence it is necessary to investigate whether meat samples being used for MFI analysis can be frozen, and which homogenisation speed and time would detect optimum differences in the fragmentation.

1.6 THE SOFTNESS OF MEAT

There has been much research on the texture of meat, especially of cooked meat. Historically, texture was not well defined and it was referred to in a very broad sense of how meat looks and feels. The word texture can encompass many properties of meat such as tackiness, tenderness, juiciness, chewiness and softness/firmness just to mention a few (Szczesniak, 1986; Halmos, 1997). Everyone knew the meaning of texture but because the term was broad, not everyone had the same definition of the word. However, as research in texture became more developed so did the understanding of texture and the number of methods available for assessing meat texture. In this review, texture refers only to the softness or hardness of meat and only this characteristic will be discussed.

Since it is the softness of raw meat that is the problem and it is what the consumers see prior to purchasing, it is important to be able to measure the softness of raw meat rather than cooked meat. Cooking causes structural changes to the meat and the cooking process also affects the softness and hence the softness of cooked meat does not represent softness of raw meat.

There are two main methods of assessing meat softness, through subjective SE or instrumental techniques. Sensory evaluation involves using a group of people (a panel) either trained or untrained to assess the softness while the instrumental techniques use various instruments to measure the desired mechanical properties of meat, e.g. softness or tenderness. The advantages of instrumental methods over SE are that it is cheaper, quicker and does not need a large group people where large variation can occur due to individual perception (Winger, 1979; Szczesniak, 1986; Halmos, 1997). It also allows quantification, that is, putting a numerical value to the parameters or properties being measured. However, the argument remains that ultimately it is the

consumers who select the products and not the instrument and therefore instrumental methods are often compared to SE to show how well the results correlate with each method.

1.6.1 Factors Affecting the Softness of Raw Meat

Animal muscles consist of four components, the myofibrillar proteins, sarcoplasmic proteins, connective tissues and intramuscular fat (marbling) which can all affect meat softness to varying extent. It is known that the myofibrils are the first major determinant of softness of raw meat followed by connective tissues and to a lesser extent, intramuscular fat (Bailey, 1972; Trevor and Bailey, 1981; Harris and Shorthose, 1988; Kuypers and Kurth, 1995).

1.6.1.1 The Effects of Connective Tissues and Marbling on Meat Softness

The effects of connective tissues on meat tenderness have been extensively researched. The amount of connective tissues differ with age, sex, breed and muscle of the animals and although the total amount of collagen decreases with age, collagen becomes tougher due to the development of cross linkages as the animals get older (Ramsbottom and Strandine, 1948; Dransfield, 1977; Bailey, 1989; Kuypers and Kurth, 1995). It has been reported that when meat is heated the collagen contracts, altering its alignment and orientation compared to the relaxed state in the raw meat, thus could greatly affect meat softness (Dransfield, 1977; Bailey, 1989; Kuypers and Kurth, 1995). Dransfield (1977) showed that the compression of cooked meat on a 7-year-old animal was 4 times greater than that of a 3-month-old. This was consistent with Bouton *et al.* (1972) who showed a high correlation between compression, tensile and Warner-Bratzler tests to the age of the animals and cooking effects. That is, older cattle has more matured collagen and hence has greater effects on the softness of meat when cooked. Connective tissue content of bovine is only about 2 - 6% expressed on dry weight (Dransfield, 1977), however its contribution to toughness is significant, and hence could play an important role in muscle softness.

It is thought that the softness of raw meat can also be affected by marbling because it can reduce the amount of myofibrillar proteins per unit volume. Since fat is softer than myofibrillar proteins, it is hypothesised that the higher the marbling, the softer the meat. The amount of marbling would also be expected to differ due to the breed and sex of the animals (Campion *et al.*, 1975). We know how strongly collagen can affect the tenderness of cooked meat but there has been little research on how the maturity and amount of collagen affect the softness of raw meat (Wood,

1991). If collagen and marbling can significantly affect the softness of raw meat then it would be expected that the softness would be influenced by those factors that affect collagen and marbling.

1.6.1.2 The Effects of Myofibrillar Proteins on Meat Softness

How do differences in meat quality give rise to differences in meat softness? DFD meat is usually firmer, has higher ultimate pH and better WHC than normal meat while PSE is the softest and has lowest WHC. There is greater sarcoplasmic and myofibrillar protein denaturation in PSE meat than DFD (Judge *et al.*, 1989; Offer *et al.*, 1989; Lawrie, 1991; Offer, 1991; van Lack and Solomon, 1994; Warner *et al.*, 1997). Since sarcoplasmic proteins are suspended in the sarcoplasm and do not contribute to the mechanical structure of the muscle then it is difficult to account for the soft texture in PSE meat due to the denaturation of these proteins (Offer and Trinick, 1983; Offer, 1991; van Lack and Solomon, 1994). On the other hand, myofibrillar proteins not only hold the bulk of the water in muscle but they, and also connective tissues are responsible for the mechanical strength as well as physical structure of muscle (Bailey, 1972; Sims and Bailey, 1981; Kuypers and Kurth, 1995). The onset of rigor mortis and protein denaturation cause the myofibrillar proteins to undergo changes in filament spacing, extracellular space, shrinkage of the head of the myosin molecules, fibre diameter shrinkage and sarcomere shortening (Offer and Trinick, 1983; Monin and Laborde, 1985; Offer and Cousins, 1992; van Lack and Solomon, 1994). Consequently these changes affect the amount of water the muscle can hold.

It is more likely that the denaturation of myofibrillar proteins and shrinkage in the lattice account for the softness in PSE meat rather than the denaturation of sarcoplasmic proteins. In addition, it has been suggested that the contraction state of the myofibrils can influence the orientation and alignment of the connective tissues. In a contracted muscle, the orientation of collagen fibres relative to the muscle fibres can change which alters the number of collagen fibres per unit area of the muscle (Trevor and Bailey, 1981). Therefore, it is possible that the connective tissues and myofibrillar proteins can directly or indirectly affect the texture of raw meat more than we can account for at present. Currently, there is very little research that explains why PSE meat is soft and whether there is always a link between softness of raw meat and the amount of exudate. There is also very little research that relates the muscle's structural changes to its softness.

1.6.2 Sensory Evaluation of Softness

Currently, research and discussions on SE of softness of raw meat is very limited compared to SE of cooked meat texture such as tenderness and juiciness. Despite this, much of the discussion of SE of cooked meat texture is also relevant to SE of raw meat. The following will discuss two important aspects of SE, which are trained or untrained panel and the scoring system. Also some of the difficulties involved in SE will be discussed.

1.6.2.1 Trained or Untrained Panel

There are two categories of SE panels, 1) the untrained, usually the consumers are selected randomly, and 2) the trained laboratory panels (Winger, 1979; Szczesniak, 1986). Untrained consumer panels are usually used to evaluate product acceptability or simple difference tests where the result is meant to reflect the common consumer perception of people who are not experts in the field. This could lead to significant variation in the valuation scores and hence a large number of people are required which makes it expensive to conduct (Hunt and Hedrick, 1977; Winger, 1979; Szczesniak, 1986; Beilken *et al.*, 1991; Halmos, 1997).

On the other hand, a trained panel, if used regularly as an analytical tool can carry out complex and detailed evaluations. The panels have to be screened for their ability to detect and differentiate the attributes of the meat being assessed. This is done by either using a triangle system where two of the three samples are the same or a scoring system to show how consistent are the scores the judges. The scoring system is usually preferred for meat since it is extremely difficult to find the same meat two pieces. Once selected the panels must be trained over the entire range of variation of the attribute in the test procedures. In addition the individual's sensitivity must be improved and the performance of the panels must be monitored regularly (Winger, 1979; Szczesniak, 1986). Approximately ten or more people would be considered a good number for a trained panel.

1.6.2.2 Scoring System

There are commonly two scoring methods for SE, a point scoring system and bipolar bar. The point scoring system commonly used consists of an eight point scale with words to describe each score from one extreme to the other, for example, 1 = extremely soft and 8 = extremely firm (Winger, 1979; Szczesniak, 1986; Trout, 1992a). This system usually does not have a mid-point

to avoid panels commonly choosing the neutral point due to their indecision. The bipolar bar method consists of a continuous line bar with no numbering with the extremes of the scale anchored with a description. The panels score by marking along the continuous line and its distance from either end of the line is measured (Raffensperger *et al.*, 1956; Szczesniak, 1986).

1.6.3 Instrumental Methods for Assessing the Softness of Raw Meat

The literature contains many methods to measure different mechanical properties of meat, and these include elasticity (multiple compression test), cohesiveness (tensile test perpendicular to fibre direction), tenderness (shear and tensile tests) and softness (compression test) (Friedman *et al.*, 1963; Stanley *et al.*, 1971; Bouton and Harris, 1972; Sale *et al.*, 1984; Harris and Shorthose, 1988; Lepetit and Culioli, 1992). Of these, the shear and compression methods have been the most extensively investigated and reviewed. Since softness of meat is the property of interest, only the compression test will be discussed here.

A compression test measures the force required to compress a sample to a given level, the greater the force the harder is the sample. Friedman *et al.* 1963, developed a two-cycle compression method for various foodstuffs using different plunger diameters (5/8 - 1/2 inches) to compress a standard sample height from 1/2 to 1/8 inch. The samples were compressed on the same spot to generate two peaks. The height of the first peak measured the softness of the samples and the combinations of these two peaks were used to calculate secondary parameters such as cohesiveness, adhesiveness and elasticity. This method, known as texture profile analysis, TPA, currently has wide application for foods.

Bouton *et al.* (1972) adopted this method using a 63 mm diameter, flat-ended plunger to compress 80 mm into a 100 mm thick slab of meat in which the muscle fibre ran parallel to the cut surface and perpendicular to the compression direction. The parameters measured were the same as described by Friedman *et al.* (1963). This method used the softness of cooked meat as measured by compression as a means of investigating the effects of cooking temperatures, connective tissues and ageing. Cooked meat is quite firm and allows accurate cutting of the samples into specific dimensions. However, Bouton *et al.* (1972) did not investigate whether this compression test could be used to measure the softness of raw meat.

Sale *et al.* (1984) devised a compression test that has a cell fitted with two lateral walls so that the sample deformation is limited to one direction. The meat sample size was 1.5 cm length, 1.0 cm width and 1.0 cm height and the compression ratio was 20 - 80%. Lepetit *et al.* (1986) showed that this method was sensitive enough to detect and differentiate changes in compression force of pre-rigor raw beef through to 22 days aged raw meat at 20% compression ratio in the longitudinal direction. At this compression ratio, the maximum force increased rapidly pre-rigor and then exponentially declined post-rigor over the ageing time which resembled that of an ageing curve. They suggested that the maximum compression force at 20% ratio is measuring the integrity of the acto-myosin complex and supported their result by a high correlation with the biochemical index of ageing, measured by Ca-Mg activated ATPase activity at different KCl ionic strengths over the ageing time. The Ca-Mg activated ATPase activity gives an indication of the integrity of the acto-myosin complex (Ouali, 1984; Ouali, 1990).

If this method is sensitive enough to monitor the development of rigor by measuring differences in compression force and is highly correlated to the biochemical index of ageing, then it appears to be the most suitable method for measuring the softness of raw meat. However, there are still many questions remaining. The sample size used in the compression was very small and thin such that the container walls and base would be expected to affect the sample's true softness. Furthermore, it is questionable whether the compression test measured on such a small sample be representative of the softness of the whole muscle. However, there is a limit to the size of a sample which can be analysed using an instrument. A correlation of the compression test with subjective SE on the whole muscle with different softness would be very useful.

Current compression tests are conducted perpendicular to the muscle fibre, and it has been reported that this measures the myofibrillar and connective tissue contribution to softness whereas compression along the grain only measures the connective tissue resistance (Bouton and Harris, 1972; Lepetit et al., 1986; Harris and Shorthose, 1988; Lepetit and Culioli, 1992). From personal observation, this appears to be true since muscles pressed across the fibre are a lot firmer than along the fibre. However, softness is a multi-dimensional property and it therefore could be argued that the softness of the whole muscle is limited to its softest side. As a result, a better measure of the softness is perhaps to compress the muscles along the fibre and not across as is commonly being done.

From personal observation, the above methods require the muscle to be cut accurately into specific dimensions, but since raw meat, especially a very soft one, is so easily stretched or compressed, these dimensions could be extremely variable leading to significant error or variability in the results. The degree of accuracy in cutting to these specific dimensions is extremely time-consuming such that when there are many samples to analyse it becomes impractical. Locker and Daines (1975) reported that muscles held at 37 °C pre- and post-rigor showed distinct softness where the fibre bundles tended to slide over each other and a cube of meat tended to flop into a rhomboid and accurate transverse cutting became difficult. Therefore, instrumental methods that will differentiate softness of raw meat and avoid the difficulties of having to accurately cut the muscles into specific dimensions are greatly needed. Most softness assessment of raw meat is done through SE and most of the existing instrumental methods are suitable for cooked meat. There is a need for instrumental methods to assess the softness of raw meat to avoid problems that are inherent of SE.

1.7 CONCLUSION

In order to study the softness of raw meat, it is essential that an instrumental method is developed to quantify muscle softness without having to rely only on a SE panel. An instrumental method for quantifying meat softness may overcome the expense, the training required and the subjectivity of a SE panel. However, the current instrumental methods available for measuring meat softness are mainly suitable for cooked meat. Although a few methods are being used for compression of raw meat, these methods are limited to very small sample sizes and require very accurate cutting of the meat samples into the desired dimensions. It is very doubtful that the very small samples being compressed will be able to measure softness without the interference of the supporting base and container walls. Therefore an instrumental method that will measure muscle softness on larger pieces of muscles and does not require accurate cutting of the samples needs to be developed.

In summary, of the factors that affect the WHC of muscle, protein denaturation and structural changes due to rigor mortis are the two most influential. The proteins being denatured are the sarcoplasmic and myofibrillar proteins and this can be measured by reduced protein solubility and myofibrillar ATPase activities. The denaturation of myofibrillar proteins causes shrinkage of the head of the myosin molecules and also shrinkage in the fibre diameter. On the other hand, the inevitable onset of rigor mortis causes reduction in filament volume due to cross bridging and overlapping of the myofilaments. Furthermore, subsequent changes such as shortening, reduction in lattice spacing, increasing extracellular space and the fall in pH will cause loss of WHC to a varying extent.

Protein denaturation and structural changes post rigor are affected by both the rate of pH decline and low pH/high muscle temperature interaction. In addition, many factors such as electrical stimulation, animal factors, the muscle temperature and the muscle fibre type have a major influence on the rate of pH decline and hence make it difficult to predict the different pH decline characteristics. So far it has been shown that the higher the temperature and the faster the pH decline, the greater will be the protein denaturation, the loss of WHC and the amount of drip. A thorough understanding of how these factors exactly influence the rate of pH decline and the interaction of pH and muscle temperature may enable a more effective control of the conditions causing excessive loss of WHC and increased drip.

The effects of proteolysis on the WHC of meat are not well understood at present. However, if proteolysis can significantly increase tenderness in the early period of ageing by degrading the muscle proteins and disrupting their structural integrity, then the ability of the muscles to retain fluid would not be as high as the intact form. Is there a significant relationship between proteolysis and the WHC of aged meat, and if there is, then how will the various rates of pH decline and high temperature/low pH interaction affect proteolysis?

Conditions causing low WHC and high drip often result in softer meat as in the case of PSE. Hence, is it possible to conclude that low WHC is the cause of soft meat and therefore softness is influenced by biochemical processes that affect muscle proteins? If so, then it is necessary to quantify these effects. In addition, it is postulated that physical factors such as connective tissues and intramuscular fat also affect softness. Hence softness can be affected by many factors and it is difficult to determine how much each factor contributes. Furthermore, in order to compare the effects of biochemical and physical factors on softness, the softness must be measured. Currently, there are no suitable instrumental methods that can achieve this purpose.

At present, the exact mechanism underlying the control of excessive water loss and softness in meat is unknown, but significant understanding of what causes high water loss has been made. So far the watery meat problem can be minimised by reducing protein denaturation, muscle shortening and abnormally high fibre diameter shrinkage due to the rapid pH decline and high temperature/low pH interaction. Appropriate understanding of the major influential factors such as electrical stimulation, animal factors, the muscle temperature and the muscle fibre type with appropriate chilling could make this possible.

1.9 HYPOTHESES AND AIMS

The hypotheses are;

1) Freezing of meat and homogenisation conditions will affect the MFI values and quantitation of the effects and implementation of standardised procedures in the assay will improve the accuracy and repeatability of the assay.

2) An instrumental method can be developed to quantitate raw meat softness which will correlate with consumer assessments of softness.

3) Soft texture in beef muscles is mainly due to insufficient control of the pH and temperature decline and accelerated proteolysis may result in early development of softer muscles.

4) High muscle temperature leads to softer muscles and soft textured muscles can have high WHC.

Thus the aims of this research were to:

1) Refine the MFI method and investigate the effects of freezing samples.
2) Develop an instrumental method to assess the softness of raw meat which will predict sensory panel scores for meat softness.
3) Investigate the relationship between WHC and muscle softness.
4) Investigate how the pH and temperature decline rates affect protein denaturation, sarcomere shortening and proteolysis, and subsequently the WHC and softness of beef.

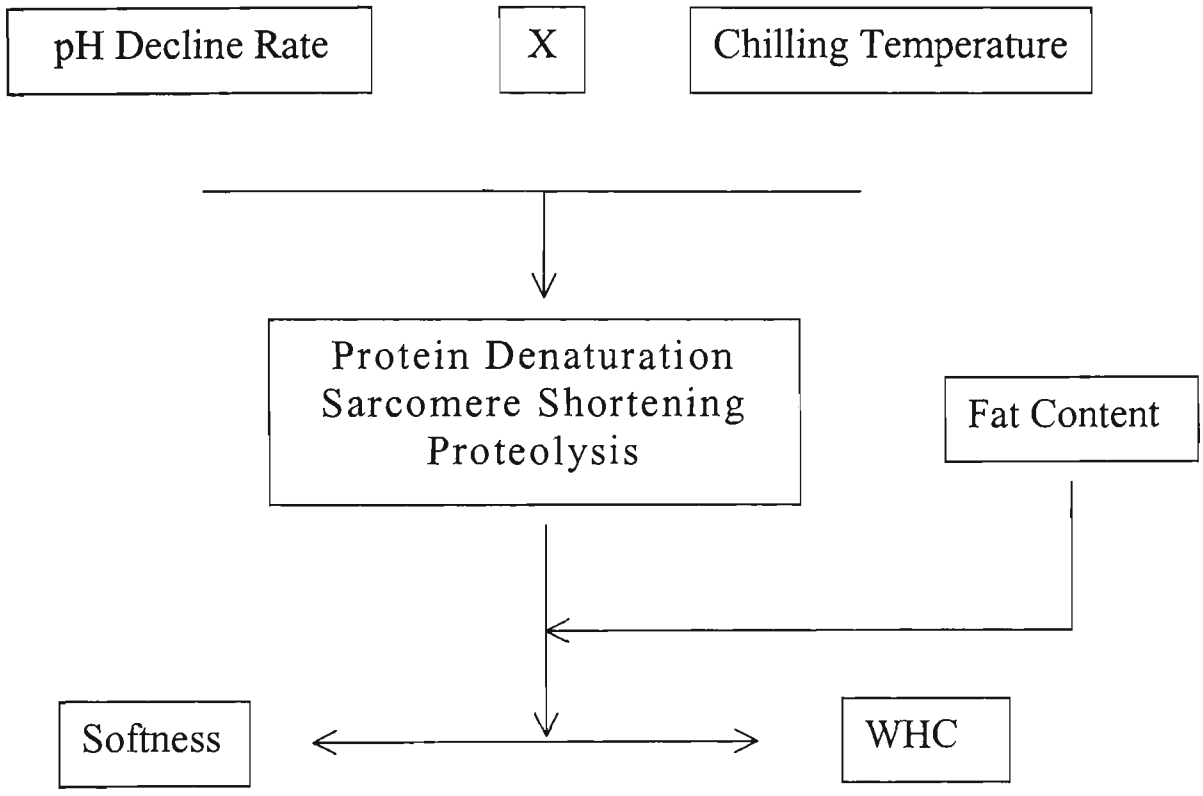
pH Decline Rate
X
Chilling Temperature
Protein Denaturation
Sarcomere Shortening
Proteolysis
Fat Content
Softness
WHC

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Figure 1.1 : Diagram of the organisation of skeletal muscle from the gross structure to the molecular level (Judge, *et al.*, 1989).

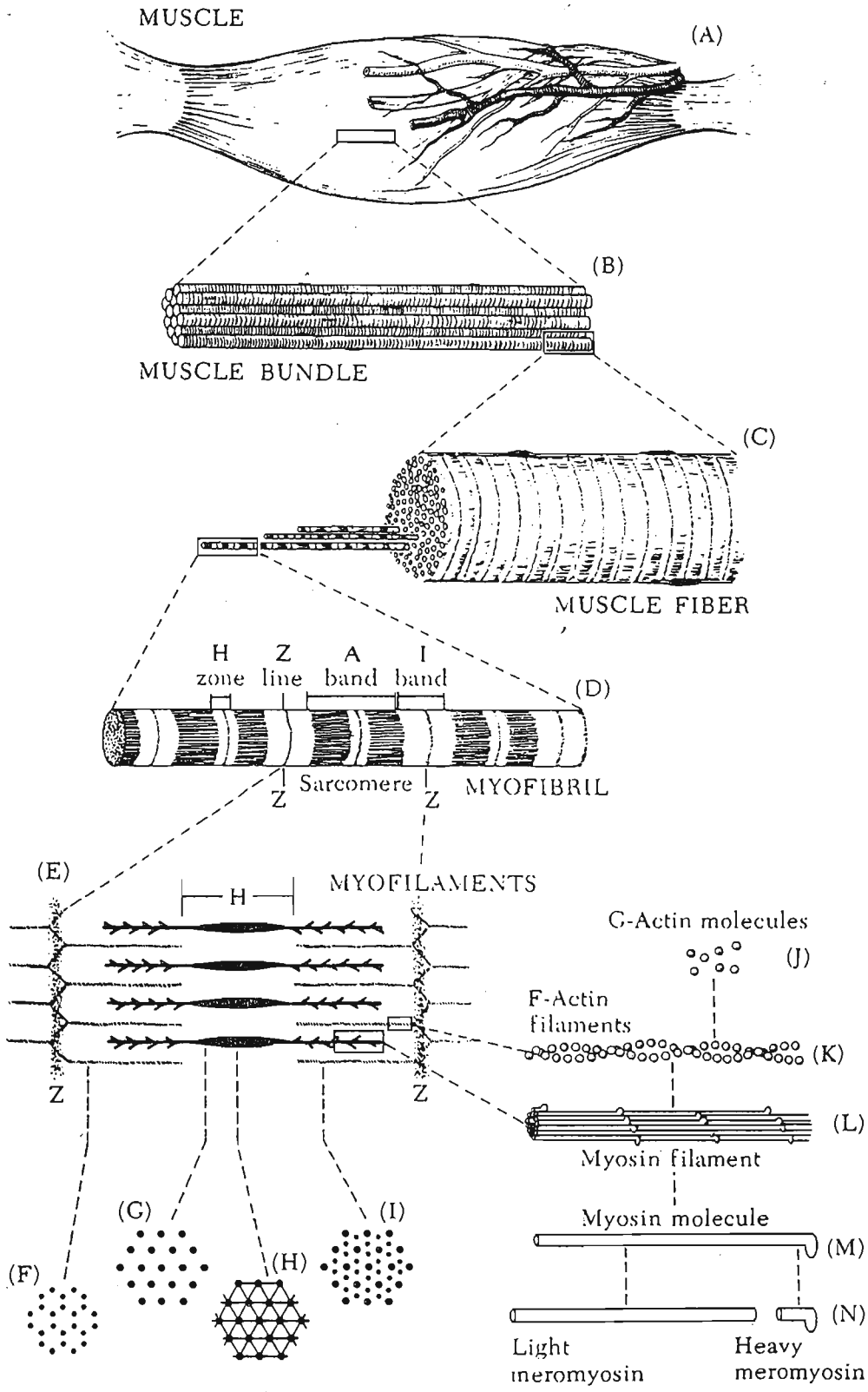


Figure 1.2: Diagram showing the location of different myofibrillar proteins within the sarcomere (Pearson and Young, 1989).

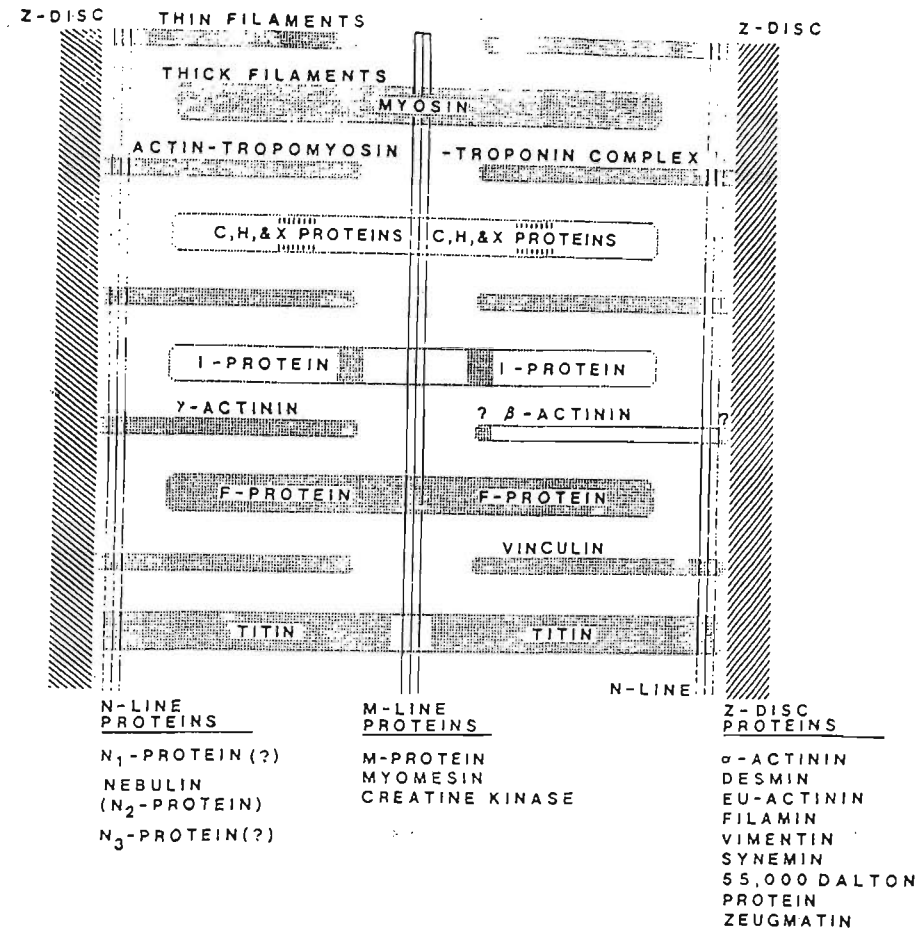
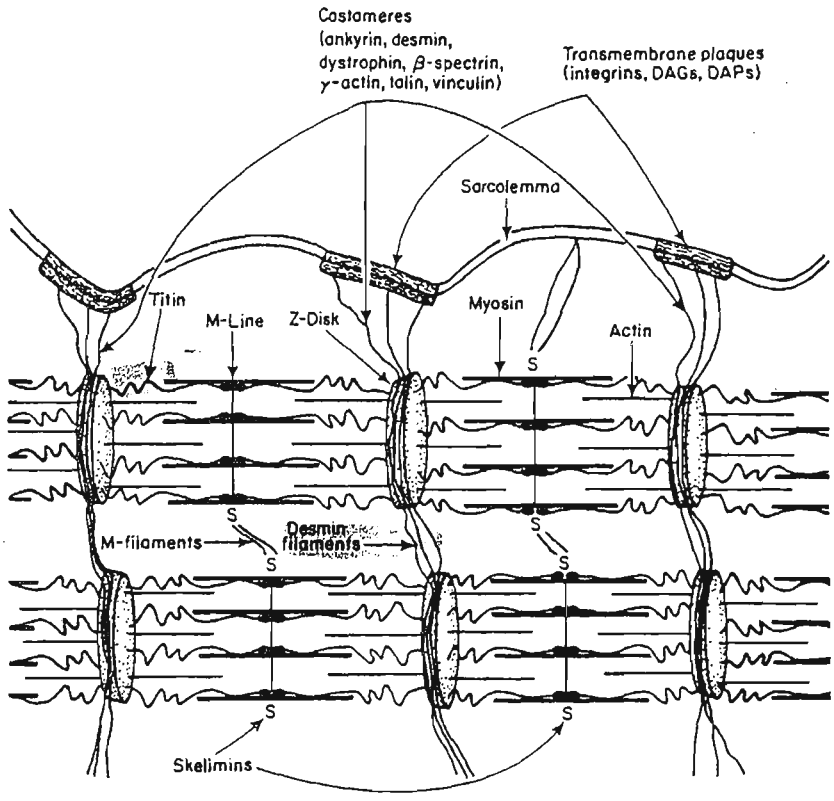


Figure 1.3 : A simplified diagram showing the structure and protein composition of costameres in muscle relative to the Z-disks and the myofibrillar lattice (Taylor, *et al.*, 1995).



CHAPTER 2

2.0 DEVELOPMENT OF THE MYOFIBRILLAR FRAGMENTATION INDEX AND INSTRON COMPRESSION FOR MEASUREMENTS OF MUSCLE SOFTNESS

2.1 THE MYOFIBRILLAR FRAGMENTATION INDEX

2.1.1 Introduction

MFI is used as a measure of post-mortem proteolysis in muscle tissue. The method measures how easily muscles break into small fragments upon homogenisation by measuring the absorbance of a solution containing a fixed concentration of homogenised muscle cell suspension. The higher the absorbance is, the smaller are the myofibrillar fragments. The theory is that tender meat should break into much smaller fragments compared to tougher meat and thus the MFI could be used to measure meat ageing or proteolysis (Davey and Gilbert, 1969; Olson *et al.*, 1976).

The MFI results can be affected by both the homogenisation speed and time of homogenisation (ie. the amount of homogenisation) for the particular homogeniser being used (Davey and Gilbert, 1969; Olson *et al.*, 1976). Olson *et al.* (1976) showed that there was a rapid increase in the MFI values at short homogenisation times between 10 to 30 secs and then very little increase after 40 secs for LD samples from day 1 and 7 post-mortem. Olson *et al.* (1976) showed that even with excessive homogenisation, the MFI value for the day 7 samples was much higher than the day 1. However, the author believed that excessive homogenisation of the muscles would result in little differences in the MFI values between aged and unaged meat, and thus make it difficult to detect differences in the MFI due to treatments applied to the muscles. Culler *et al.* (1978) and Koohmaraie *et al.* (1996) both reported a maximum MFI of 60 for their most tender samples while Olson *et al.* (1976) and Whipple *et al.* (1990) reported a maximum of 76 to 80 respectively. This shows that the MFI values vary between and within research groups even though their methods may be very similar. It is possible that this is due to differences between the samples.

Some of the MFI results that have been reported in the literature were analysed from frozen samples (Moller *et al.*, 1973; Culler *et al.*, 1978; Bruce and Ball, 1990) while others were from fresh samples (Davey and Gilbert, 1969; Olson *et al.*, 1976; Whipple *et al.*, 1990; Koohmaraie *et al.*, 1996). There has been no discussion in the literature of how freezing of meat would affect the

MFI results. Perhaps freezing has negative effects on the muscle structure and thus possibly on the MFI results. An advantage of being able to freeze the samples would mean that the assay could be done at a later date.

The hypotheses are that (i) freezing of meat, homogenisation time and homogenisation speed will affect the MFI values and (ii) quantitation of the effects and implementation of standardised procedures in the assay will improve the accuracy and repeatability of the assay.

The aims of this experiment were to investigate the effects of homogenisation time and speed and freezing samples on the MFI results.

2.1.2 Materials and Method

Eight beef striploins were selected from a local boning room at random. Four of the eight striploins had been aged 1 day and the other four for 14 days. The history and carcass specification of the samples was not recorded. These loins were transported to the laboratory on ice where each loin was cut in half, one half was vacuum packaged and frozen at –20 °C and the other half analysed fresh. The MFI method of Olson *et al.* (1976) as modified by Culler *et al.* (1978) was used with variations given below.

The frozen samples were analysed on a different day to the fresh and were thawed by immersing in cold tap water for about 5 mins before finely chopping them in a semi frozen state. For each sample (days 1 and 14, fresh and frozen), approximately 20 g from a cross section of the loins representative of the whole piece, free of visible fat and connective tissue was finely chopped. Duplicate 2.0 g samples were added to 20 mL cold (2 °C) MFI buffer (100 mM KCl, 20 mM potassium phosphate (pH 7.0), 1.0 mM EGTA, 1.0 mM $MgCl_2$, and 1.0 mM NaN_3) and homogenised with a Waring blender (model 32RL80, USA). Two different homogenisation speeds (high and low) and three different times were used. For the low speed, 30, 60 and 90 secs of homogenisation was used while for the high speed, 15, 30 and 60 secs of homogenisation was used.

Subsequently all samples were centrifuged at 1000 G for 15 mins at 2 °C, the supernatant discarded, but the fat cap (layer of fat, connective tissue and small amount of myofibrils) above the supernatant was retained with the pellet. The pellet and fat cap were re-suspended in 20 mL

of cold MFI buffer using a flat-ended stirring rod, centrifuged as before and the supernatant and fat cap discarded. The pellet was re-suspended in 10 mL of cold MFI buffer, vortexed until well mixed and filtered through a polyethylene strainer (mesh size 8) to remove connective tissues. The centrifuge tube was rinsed with an additional 10 mL MFI buffer and poured through the polyethylene strainer.

The protein concentration of the suspension was determined by adding 3.0 mL biuret reagent (0.036 M CuSO4, 0.127 M sodium potassium tartate, 3% (v/w) NaOH) to 0.25 mL of the myofibrillar suspension diluted to 1.0 mL with distilled water (Gornall *et al.*, 1949). Bovine serum albumin (BSA) in the concentration range of 0 – 1.25 mg/mL was used as standard and the absorbances of both the samples and standards were read at 540 nm after 30 mins. After the concentration was determined, the myofibrillar suspension was diluted to 0.5 mg/mL with MFI buffer to a final volume of 8.0 mL . The mixture was gently inverted 3-4 times to mix, immediately poured into a micro cuvette and the absorbance read at 540 nm. The readings were taken where the fluctuating value was most stable and duplicate readings were taken from each 8 mL of 0.5 mg/mL solution. The MFI values were calculated by multiplying the absorbance by 200 to obtain whole numbers as described by Olson *et al.* (1976).

The data was analysed for differences between homogenisation time, speed, ageing day and fresh or frozen by analysis of variance (ANOVA) using SPSS (version 9) statistical software (SPSS Inc. Chicago, Illinois, USA). The experiment was balanced with equal number of samples being analysed for each treatment.

2.1.3 Results and Discussion

Homogenisation Time and Speed

Table 2.1.1 shows the effects of homogenisation speed and time and ageing days (day 1 vs day 14) on the MFI values of beef LD muscles. For day 1 samples, increases in the homogenisation speed and also time resulted in higher ($P<0.001$) MFI values. The MFI value of 41 for day 1 samples homogenised at low speed for 30 secs was consistent with the results of Olson *et al.* (1976) who reported MFI values of about 45 for day 1 LD beef muscles using a similar method. For day 14 samples, increased homogenisation time gave higher ($P<0.001$) MFI values but there was no difference due to homogenisation speed ($P>0.05$). The MFI values of day 14 samples

were all above 80 for all treatments and increased very little with increasing amounts of homogenisation. This indicates that the aged meat was readily broken into smaller fragments and quickly reached a high fragmentation level with little change thereafter. The MFI values of just above 80 for the day 14 fresh LD was also consistent with the reported range of 70 to 80 for fresh beef LD at day 7 with homogenisation time greater than 30 secs from Olson *et al.* (1976) and Whipple *et al.* (1990).

Homogenisation with low speed for 30 secs (LS30) or high speed for 15 secs (HS15) resulted in the largest difference in MFI between the day 1 and 14 samples. When the amount of homogenisation was increased by either speed (high) or (time) the values for the difference in MFI values between day 1 and 14 diminished ($P>0.05$). As speculated, excessive homogenisation did lead to only small difference in MFI between the aged and unaged samples and hence did not support that of Olson *et al.* (1976) who found that even with excessive homogenisation, there was still a large difference between the aged and unaged samples. Although HS15 gave lower MFI than LS30 ($P<0.05$), the difference in MFI between day 1 and 14 was similar for the two methods ($P>0.05$). Both of these methods (HS15 and LS30) are suitable for the MFI analysis since they showed the greatest difference in MFI between day 1 and 14. However, the LS30 would be a more favorable method because with the high speed homogenisation, 15 secs of homogenisation is more difficult to time exactly and a small error in the homogenisation time could lead to inaccuracies and variation in the MFI results.

The MFI of Fresh and Frozen Samples

Table 2.1.2 shows that the MFI values were different ($P<0.01$) due to the method (LS30 vs HS15), the days of ageing (day 1 vs day 14) and the phase of the samples (fresh vs frozen), but there was no interaction between these parameters ($P>0.05$). The MFI values of frozen samples were lower ($P<0.01$) than of fresh samples. Day 14 samples were higher in MFI than day 1 samples ($P<0.001$) and LS30 resulted in higher ($P<0.05$) MFI values that HS15. However, the MFI difference between day 1 and day 14 was not different between the methods or the phases ($P>0.05$, Table 2.1.3). This shows that regardless of the method or phase of the samples, the differences in MFI between day 1 and 14 are similar.

Culler *et al.* (1978), has been frequently cited by other authors and reports a maximum MFI value of 60 from the most tender beef LD muscles that had been aged for 10-14 days and frozen. This is in the same MFI range obtained for the frozen samples in this experiment. In addition Olson *et*

al. (1976) and Whipple *et al.* (1990) reported MFI values of 76 to 80 for fresh beef LD at day 7 which is consistent with the MFI values obtained from the fresh samples in this experiment. The results of this experiment are consistent with the reported MFI values that vary significantly depending on whether the samples were frozen or fresh.

It is a concern that the MFI values of frozen samples were significantly lower than that for the fresh samples because it could limit direct comparison between fresh and frozen. However, in the study of proteolysis in muscle, the change in MFI value is often more useful as an indicator of ageing rate than the absolute value at a specific time point. Differences in the MFI values between treatments at one time point are usually the most important when it comes to determining the effects of treatments on the muscle. Furthermore, the MFI values of the frozen meat are able to show differences between the treatments just as well as the unfrozen meat. Hence, it is recommended that samples can be frozen for MFI analysis if it is used for monitoring ageing rate, as shown by the changes in the fragmentation of the myofibrils.

2.1.4 Conclusion

It was found that unaged meat was more affected by the amount of homogenisation than aged meat and that excessive homogenisation did lead to only a small difference in MFI between the aged and unaged samples and hence justified the research into developing an optimised method. Homogenisation with the Waring blender at low speed for 30 secs or high speed for 15 secs gave the largest differences in MFI value between day 1 and day 14, and hence the two methods are suitable for studying tenderisation.

The MFI values of frozen samples were lower than fresh, however the difference in MFI between day 1 and day 14 was the same for the fresh and the frozen samples. Therefore it was concluded that samples for MFI could be frozen for future analyses if it is used for measuring the changes in fragmentation level (i.e. ageing rate) and not for the absolute values or direct comparison to MFI values of fresh samples.

2.2 DEVELOPMENT OF INSTRON COMPRESSION FOR MEASUREMENTS OF MUSCLE SOFTNESS

2.2.1 Introduction

The word texture encompasses many mechanical properties of meat such as tackiness, tenderness, juiciness, chewiness, mouth feel and softness/firmness. A large amount of research has been conducted on the texture of meat with respect to tenderness and softness of freshly cooked meat or processed foods, but not as much has been conducted on in the softness of raw meat. The softness or firmness of raw meat is an important physical property and quality defects such as PSE or DFD undoubtedly affects the marketability of meat.

Given the importance of softness, there is a deficiency of suitable instrumental methods for quantifying the softness of fresh meat when there is a problem. Currently, most softness assessment of raw meat is done by a panel of judges pressing and squeezing the raw meat to score it for softness (Winger, 1979; Szczesniak, 1986; Trout, 1992a; Halmos, 1997) and most of the existing instrumental methods are for measuring the softness of cooked meat (Bouton and Harris, 1978; Harris and Shorthose, 1988; Lepetit and Culioli, 1992; Halmos, 1997). Using people to assess the softness of raw meat is subject to individual opinion and perception of softness and hence can result in large variations between softness scores. There is often a dilemma whether to use trained or untrained panels since most customers are not well trained and would be using their own perception of what is defined as "soft" and "firm" meat. Furthermore, a relatively large number of people are required to run a sensory panel to obtain consistency in the softness scores as would be required for running a taste panel. Instrumental methods may overcome some of these problems as they are cheaper, quicker and do not need a large group of people to conduct a test. Instrumental methods also allow quantification, whereas a sensory panel is qualitative with arbitrary scores based on the perceived softness.

Using the compression method of Sale *et al.* (1984), Lepetit *et al.* (1986) were able to monitor the changes in compression force of pre-rigor meat through to 22 days aged raw meat. At the 20% compression ratio, the maximum force increased rapidly pre-rigor and then exponentially declined post-rigor over the ageing time which resembled that of an ageing curve. They suggested that the maximum compression force at 20% ratio was measuring the integrity of the acto-myosin complex and supported their result by a good correlation with a biochemical index of ageing as measured by the Ca-Mg activated ATPase activity.

If this method was sensitive enough to monitor the development of rigor by measuring differences in compression force and was highly correlated to the biochemical index of ageing, then perhaps it could also be used to measure the softness of raw meat. However, the sample size used in their compression was very small and thin (1x1x1.5 cm) such that the container walls and platform used to hold the sample would affect its true softness. Furthermore, it is questionable whether a compression test on such a small sample would size be representative of the firmness of the whole muscle. Although it was not the authors aim to investigate the softness of the muscle, a correlation of the compression test with SE would have been very useful.

Instrumental analysis of softness also has a number of inherent problems. Most of the current methods given by Bouton and Harris (1978), Lepetit *et al.* (1986), Harris and Shorthose (1988) and Lepetit and Culioli (1992) require meat samples to be cut accurately in a specific dimension (eg. either a 1.0 cm cube or 1.0 cm thick slab cut parallel to the muscle fibres). Since raw meat is so easily stretched or compressed, these dimensions can be so variable that from personal observation, it could lead to significant variability in these dimensions, and variable results. Also, the degree of accuracy required in cutting these dimensions is extremely time consuming such that when there are a lot of samples to analyse it becomes impractical.

The hypothesis was an instrumental method can be developed to quantitate raw meat softness which will correlate with consumer assessments of softness.

The aim of this experiment was to develop an instrumental method that; 1) can measure the softness of raw meat without the above problems and 2) can predict SE scores for meat softness.

2.2.2 Materials and Method

Selection of Samples for Sensory Evaluation

Four beef SM muscles were selected from a commercial abattoir. Two of the four muscles which came from an old cow (about 8-12 years old, samples I and III) were soft and dark while the other two (samples II and IV) were from normal prime beef and were bright red and firm. The samples were trimmed of all visible fat and connective tissues and randomly laid out in a room at controlled temperature of 15 °C. A group of 16 untrained judges were shown how to assess the whole muscle for softness by squeezing and pressing along and across the muscle fibre. The

scoring scale was from 1 to 8, 1 being extremely soft and 8 extremely firm as described by Trout (1992). No reference score was provided as guidance for the different levels of firmness. Subsequently each of these muscles was cut into half, with one half cut into smaller blocks of about 7x8 cm and 5 cm thick parallel to the fibre direction and the other half vacuum packaged, aged for 6 days at 2-4 °C and then cut into the same size blocks. These blocks were then randomly assessed for firmness by the same judges in the same manner as the whole muscle but giving more attention to the softness along the muscle fibre than across the muscle fibres.

Measurement of Muscle Softness Using the Instron Compression

After the judges had assessed the softness, the 7x8 cm muscle blocks were cut smaller to 5x6 cm surface area and 4 cm thick parallel to the muscle fibre direction and held on ice before analysis. The compression set up using the Instron Universal Testing Machine (model 4465, series 9, Canton, MASS) is shown in Figure 2.2.1. This compression method was adopted and modified from the method of (Bouton and Harris, 1972) who used a 63 mm diameter, flat-ended plunger to compress, at 50 mm/min, 80 mm into a 100 mm thick slab of cooked meat perpendicular to the muscle fibre direction. In this experiment, the flat-ended compression probe diameter was 1.27 cm instead (model T372-32, Instron Universal Testing Machine) to reduce the possibility of the small probe cutting into the meat samples. The compression probe was placed just in contact with the samples but not exerting any force. The meat blocks were then compressed at the speed of 50 mm/min along the muscle fibre to a depth of 20 mm. Each sample had 3 to 4 replicates of compression at different sites across the muscle surface and the loads (force) at compression depths of 5, 10 and 15 mm were impiracally analysed.

Statistical Analysis

To test for significant differences between the samples for the SE and IC values, all the parameters were analysed by ANOVA using General Linear Model of SPSS statistical software. Pearson correlation coefficients between the IC measurements and SE scores were also calculated using SPSS (SPSS Inc. Chicago, Illinois, USA).

2.2.3 Results and Discussion

Sensory evaluation

Table 2.2.1 shows the results of SE and IC of softness at 3 different depths. For the whole muscle and muscle blocks on days 1 and 6, the SE showed that samples I and III were softer than samples II and IV ($P<0.05$) as would be expected as they were selected to be soft and firm. Samples I and IV were scored as softer ($P<0.05$) than when assessed as a whole muscle. Sample II which was softer than sample IV as whole muscle was not different ($P>0.05$) when tested as muscle blocks. This change of softness scores from the whole muscle to muscle blocks for samples I and IV could have been due to the large variation in the softness scores or with in the muscle. It could also suggest there were large variations between consumers or a change in perception of softness in going from whole muscle to small muscle blocks.

There were large variations between the judge's softness scores within the sample. Some of these scores ranged from 1 (extremely soft) to 5 (slightly firm) within the same sample. This variation is large and thus the method of using untrained panel would not be able to detect subtle differences in softness or changes in softness due to ageing.

Between day 1 to day 6, sample II got firmer ($P<0.05$) while sample III got softer ($P<0.05$). It is difficult to say whether these change in softness scores were caused by ageing or due to inconsistencies in the softness scores between panel members. Since only one muscle from each quality group had changed instead of both it would be more likely due to the variation in the scores. However, if the softness of muscle blocks was not homogenous and the variation within the muscle was greater than the changes due to ageing, then it was unlikely that changes with ageing can be detected. Furthermore, from personal observations, inexperienced judges tended to score samples relative to each other on the day of evaluation and so spread the scores along the scale. As a result, aged samples on day 6 could have the same scores as day 1 because they were being compared within the group and not relatively between the ageing days. This problem is difficult to overcome since ageing studies require evaluations to be conducted on different days and the softness scores are arbitrary, not absolute like the Instron analysis.

Table 2.2.2 shows that the score for the whole muscle softness was highly correlated with the score for softness of muscle blocks for both days 1 and 6 ($P<0.01$, $r = 0.92$ and 0.97 respectively) despite the large variation in the softness scores. The score for the softness of day 1 muscle blocks

was also highly correlated ($P<0.01$, $r = 0.96$) with day 6 muscle blocks. Hence the two methods could potentially be used interchangeably.

Evaluating softness on uncut muscle would be preferred since it would be more representative of the whole muscle and therefore not biased by sampling one small region of the muscle. This would be particularly relevant if the softness was not uniform throughout the muscle. On the other hand, to assess the whole muscle may be difficult because the judges would have to thoroughly assess all areas of the muscle since they may contain both firm and soft sections. The overall softness scores would then vary depending on how the judges weigh the differences between the firm and the soft portions. This could lead to large variations and would reduce the sensitivity of the method and hence mask smaller differences. Thus assessing the softness using muscle blocks may be a little more precise since the judges only need to concentrate on a relatively smaller piece. But it would become important to assess several samples for each muscle to ensure the range in softness (if present) was represented in the samples assessed for each muscle. However, the amount of samples available may be a limiting factor.

Evaluation of muscle softness using the Instron compression

The results of muscle softness evaluation using the IC for days 1 and 6 are shown in Table 2.2.1. The loads for the 4 samples at days 1 and 6 increased as the compression depths increased. The loads at the 5 mm compression depth were not different between the 4 samples or between day 1 and day 6, but the loads at 10 and 15 mm on day 1 for samples II and IV were higher (firmer) than for samples I and III ($P<0.05$). These results agree with both the sensory panel softness scores of the whole muscle and muscle blocks on day 1. It appears that the 5 mm compression depth cannot detect differences in softness as well as compression depths of 10 or 15 mm.

On day 6, the compression loads were mostly lower than day 1 indicating softer muscles, except for sample 1. This did not agree with the sensory panel scores for day 6 samples which generally did not show softer samples. It was interesting to note that on day 6, the load of sample II at 10 mm compression depth was much lower than on day 1, and yet its loads at 15 mm were the same for both days. Also, samples III and IV were not different ($P>0.05$) at 10 mm but were different ($P<0.05$) at 15 mm. This shows that a compression depth of 15 mm may be more reliable than 10 mm. The compression loads of sample I on day 6 were strangely high and hence treated as outliers and were not compared to other results. Other parameters such as the work done (at different compression depths) or the maximum compression force were measured on the IC, but

their results were a lot more variable and had low correlations to SE ($P>0.05$), and thus were not included.

Although this compression method was adopted and modified from the method of Bouton *et al.* (1972), the results could not be compared since Bouton used cooked meat instead of raw meat to investigate the effects of cooking temperature, connective tissues and ageing. The cooking process would have greatly increased the firmness, altered the structure and reduced the elasticity of the meat. On the other hand, Sale *et al.* (1984) and Lepetit *et al.* (1986) conducted compression on raw meat as previously described in the literature review. However, their set up allowed the meat sample to deform only in one direction and the sample size was small (1.0 x 1.0 x 1.5 cm) compared to the sample size used in this experiment in which the container wall could affect its softness. The compression method of Sale *et al.* (1984) was used to measure the integrity and development of the acto-myosin complex from pre-rigor to post-rigor and not the softness between different muscles and how it would compare to SE of softness. Lepetit *et al.* (1986) found that the maximum force (40 N/cm^2) was at one day post-mortem and least at 9 days post-mortem (8 N/cm^2) which is comparable to ours here as the IC load decreased over 6 days.

Comparison of the Instron compression to sensory evaluation

Table 2.2.2 shows Pearson's correlation coefficients between the IC measurements and SE scores. The IC on day 1 at 5 to 15 mm depths were highly correlated with the SE scores of the whole muscle and muscle blocks on days 1 and 6 with correlations greater than 0.92 ($P<0.01$). One reason for such a high correlation could be due to the extreme differences in the four SM muscles, where two were quite soft and the other two a lot firmer. Hence it was easy to differentiate the two groups. Since the IC loads of sample I on day 6 were extremely high, its correlation to other parameters was also affected and hence was excluded.

The SE judges assessed meat softness by touching and squeezing the samples and then give an overall arbitrary softness score according to their perception. These scores are only relative and the absolute softness is unknown. On the other hand, the IC quantitatively measures the load applied to the samples in a one-dimensional compression. These two methods are evaluating softness in a different way but in order to be useful must give a similar result. Hence the two methods must be highly correlated.

Unlike SE, the IC is limited to compression in one direction, either along, across or perhaps diagonally across muscle fibre direction. Current compression tests (Bouton and Harris, 1978; Lepetit *et al.*, 1986; Harris and Shorthose, 1988) are perpendicular to the muscle fibre. It has been reported that this measures the myofibrillar and connective tissue contribution to firmness, but compression along the fibre direction only measures the connective tissue resistance (Bouton and Harris, 1972; Lepetit et al., 1986; Harris and Shorthose, 1988; Lepetit and Culioli, 1992). However, softness/firmness is a multi-dimensional property and therefore it could be argued that the firmness of the whole muscle is limited to its softest side or dimension. As a result, measuring the softness of meat by compressing along the fibres and not across is perhaps a better method since this is the softest direction.

The difficulty in trying to detect changes in softness due to ageing is that once the meat samples are used for compression on day 1 the meat is damaged and hence can not be used on another day. Therefore a separate piece would have to be kept for ageing studies, but since softness is not homogenous throughout the muscles it is difficult to know whether the difference in softness is due to ageing or merely due to variations that already existed within the muscles. As a result, sampling can also greatly affect the IC results obtained. Since the samples are cut into smaller blocks of 4 cm thick with approximately 5x7 cm surface area, then if taken from a softer section of the whole muscle it would show that the sample is soft.

If those sample blocks contain both soft and firm sections, then the overall average will depend on how many compression replicates are conducted on the soft and firm sections. Therefore, the number of compression replicates on each meat block must be in proportion to the amount of soft and firm areas of the sample to avoid bias. For example, if 30% of a meat block is soft and 70% is firmer, then only 1 out of 3 of the compression replicates should be on the soft area and 2 out of 3 on the firm area. Consequently, both of the sampling and compression steps are open to human judgement error and bias. The problems are difficult to avoid since this compression method and most of the current ones (Bouton and Harris, 1972; Lepetit *et al.*, 1986; Lepetit and Culioli, 1992) all require sampling a small amount of meat from the whole muscle, and if the softness of the whole muscle is not homogenous then the method is already open to variations even before the compression step. However, sampling variation can either be minimised by taking more readings at different locations within the same muscle, or by taking readings from the same location within each muscle but from a greater number of animals.

The samples used for the IC test were quite large (5x7x4 cm) to minimise external influences such as the effects of the meat edges and the base supporting the meat. In the IC method, the probe was placed in contact with the meat samples at the start of the test and then the load was measured at different compression depths into the samples. The compression depth for all the samples was always the same regardless of the sample height. This avoided the need to accurately cut meat into a specific dimension in order to perform a compression ratio based on knowing the exact thickness of the samples.

2.2.4 Conclusion

The three SE tests all showed that the muscles from prime beef were firmer than the muscles from the old cow. However, the SE and IC did not detect difference in softness within the muscles of the prime beef or the old cow. Despite large variations in the scores of each sample within the judges, the softness of whole muscle was highly correlated with the muscle blocks which suggested that the two tests may be used interchangeably.

The three IC depths all showed the same trend as the SE and there were high correlations between the IC and SE. However, the IC also did not detect a difference in softness within the muscles of prime beef or the old cow. These results were promising and indicated that the IC method could differentiate extreme differences in the softness of raw meat. This warrants further investigation using muscles from similar grade of cattle which have undergone treatments to affect the softness of the muscle.

At this stage it is too early to conclude whether the IC of the whole muscle is better than the muscle block or whether analysis on day 1 is more suitable than day 6. So far the IC results indicate that compression depth at 10 and 15 mm can detect differences in muscle softness.

2.3 INVESTIGATION OF THE USE OF INSTRON COMPRESSION AND SENSORY EVALUATION TO EVALUATE DIFFERENCES IN MUSCLE SOFTNESS DUE TO TEMPERATURE AND pH DECLINE RATES ON THE SOFTNESS OF BEEF MUSCLES

2.3.1 Introduction

The results of the previous experiment showed that the IC could differentiate extreme differences in the softness of raw meat and had a high correlation with SE scores of softness. However, it was suspected that the high correlation could have been caused by the fact that there were two very soft (from old cow) and two firm muscles (from prime beef) that made the softness evaluation much easier. The SE and IC did not detect difference in softness within the two very soft or the two firm muscles which could mean that the method was not sensitive enough or perhaps there was no difference in softness within the prime beef or within the old cow.

The hypothesis was that manipulating the rate of pH and temperature decline post-slaughter will affect the raw meat softness and the resulting samples can be used to evaluate instrumental methods for measuring softness against sensory evaluation of softness.

The aim of this experiment was to evaluate an instrumental method of measuring raw meat softness against sensory evaluation using SM and LD muscles of similar quality grade with a range in softness induced by applied treatments.

2.3.2 Materials and Methods

Animals and Treatments

For a detailed method please refer to Chapter 4. Briefly, twenty carcasses (weight ~ 182 kg) were ES using low voltage at 3 mins post-mortem for either 10 or 40 secs. The sides were split and either chilled rapidly at 1 °C for 10 h or slowly at 18 °C for 2 h and then at 6 °C for 8 h and then chilled at 2-4 °C until the next day. This was to obtain large differences in pH and temperature decline rates. The temperature and pH decline rates were measured on the LD and SM muscles and modelled using $X(t)=X\infty + (Xo - X\infty)e^{-Kt}$, where K is the decline rate constant.

Sensory Evaluation

At 24 h post-mortem, the LD and SM muscles were trimmed of surface fat, numbered and the LD and SM placed separately in a room chilled at 4 °C for softness assessment by 10-15 untrained judges. The judges were shown how to assess the whole muscle by pressing and squeezing. Each judge gave an overall softness score on a scale of 1 to 8 (1 = extremely soft, 8 = extremely firm). The muscles were then cut into smaller blocks approximately 5x6x8 cm. The softness of the muscle blocks was reassessed by the judges on the same day, approximately 28 h post-slaughter.

Instron Compression

After the SE, the muscle blocks were further cut into approximately 5x7x4 cm along the fibre direction for the SM and at an angle to the fibre direction for the LD (see Figure 2.3.1) to be used for the IC. The samples were kept on ice in an ice container while each was being analysed. The SM and LD muscles were compressed at approximately 30 h post-slaughter as shown in Figures 2.2.1 and 2.3.1 respectively to a compression depth of 20 mm using the same procedure as described in the previous experiment, with compression forces analysed at depths of 5, 10, 15 and 19 mm. The 19 mm compression depth was also analysed to see whether it would be better at detecting difference at higher compression depth.

To investigate the effect of ageing on softness, approximately 800 g blocks of the SM muscles were vacuum packaged, aged for 14 days at 2-4 °C and then cut into the same size blocks as day 1 for measurement of softness by SE and IC.

Statistical Analysis

To test for significant differences between the treatments (ES, Chill rate, muscle and relevant interactions) all the parameters were analysed by ANOVA using General Linear Model of SPSS statistical software. Pearson correlation coefficients between the IC measurements and SE scores were also calculated using SPSS (SPSS Inc. Chicago, Illinois, USA).

2.3.3 Results and Discussion

Sensory Evaluation

The SE of whole muscle (Table 2.3.1) showed that the LD and SM muscles were firmer with rapid chilling (score 5.0) than slow chilling (score 4.2, $P<0.001$). The difference in softness between rapid and slow chilling was greater in the LD than SM muscle (muscle x chill rate

interaction, P=0.002), but overall the SM muscles were firmer than the LD (P=0.071). When the muscles were cut into smaller blocks the results were generally similar to those for the whole muscle. The judges scored the SM muscles as firmer (score 4.4) than the LD (score 3.3, P<0.001) and rapid chill samples were much firmer (score 4.5) than slow chill (score 3.2, P<0.001). As an observation, the muscle blocks appeared to be softer compared to the whole muscle and this is demonstrated in the mean sensory scores. The assessment of softness using the whole muscle seemed to detect more significant differences in softness due to treatment effects such as ES and muscle x chill rate interaction than the muscle blocks. The whole LD and SM muscles on day 1 and the muscle blocks on day 1 showed that ES had no significant effect (P>0.05) on the softness.

After 14 days of ageing, the SM muscle blocks were softer than day 1 (P<0.001, statistical results not presented). However, the differences in softness due to chill rate or ES which were present at day 1 were not observed on day 14. There are 2 reasons that may account for these results. 1) The judges for day 14 SE were different from the day 1 since the assessment on day 14 was conducted at a different location, and 2) ageing from day 1 to day 14 may bring all the samples to a similar level of softness.

The softness score for the whole LD muscle was correlated to the muscle blocks (P<0.05, r = 0.51) but the score for the whole SM muscle did not correlate with the score for muscle blocks (P>0.05, r = 0.05). It is a concern that the softness of the whole muscle did not show a high correlation with the muscle blocks for both the SM and LD since they were derived from the same samples. This could suggest that the SE method needs to be refined, or perhaps the softness of individual muscle blocks was different to the whole muscle. Although not tested, it was observed that the anterior end of the LD muscles was softer than the posterior end. Hence if some of the judges tended to assess the overall muscle while others concentrated only on the anterior end then there could have been some variation as a result. However, since all the anterior end of the LD muscles were presented to the judges, this variation should have been minimised.

The reasons for the observed differences in going from whole muscle to blocks could be due to the appearance of the two muscles. The SM muscles were big and round whereas the LD muscles were longer, flatter and thinner, and this may have influenced the judges' perception of softness. Since the LD muscles were long and the softness was not uniform along the length of the muscles, an overall softness score may have been variable. As discussed in experiment 2.2, assessment of whole muscle can be difficult since they are large and may not contain uniform softness. When

the muscles were cut into similar sizes, these apparent physical differences may have been eliminated and hence made it easier and perhaps more precise to assess the softness. However, the smaller blocks could also lead to sampling bias.

Instron Compression

Table 2.3.2 shows that the IC force at all 4 depths (5,10,15,19 mm) were higher for the LD than the SM muscle. For the 10 mm compression depth, rapid chill resulted in firmer LD than slow chill, but there was no difference in softness between chill rates for the SM (muscle x chill rate interaction, $P<0.05$). For the day 14 samples, there was also no difference in softness due to ES or chill rate ($P>0.05$, Table 2.3.4).

The IC of the SM is parallel to muscle fibre while the IC of the LD is at an angle to the muscle fibre. This was necessary as it was difficult to obtain enough LD samples to for compression parallel to the fibres. Hence this could bias the comparison of the IC measurements between the SM and LD muscles.

One difficulty encountered was that the LD muscles collapsed under their own weight because they were soft. The muscles became more compact and also lost some of their initial softness and height. Since the compression probe still had to be placed in contact with the samples at the start of a test in order to measure the load at specific compression depths, the samples were firmer than if they had not collapsed. This resulted in variations in the results and hence caused the method to be less sensitive.

The IC was not suitable for measuring extremely soft muscles that can not maintain their shape when positioned up right and therefore would only be suitable for those samples that do not exhibit this problem. If samples could be prevented from collapsing then the problem may be avoided. One solution may be to put them in containers, although the effects of the container wall on softness would need to be assessed.

Comparison of Sensory Evaluation to Instron Compression

As reported above, the SE detected more differences in softness due to the treatments in the LD and SM muscles than the IC method. Table 2.3.4 shows that for the LD muscle, there was a high correlation between the SE of the muscle block and the IC at 10 and 15 mm depth respectively ($P<0.01$, $r = 0.79$ and 0.75). There was also a correlation ($P<0.01$, $r = 0.50$) between the SE of the

whole LD muscle and the IC at 10 mm depth. For the SM muscle, only the IC at 15 mm depth on day 1 was correlated ($P<0.05$, $r = 0.35$) with the SE of the whole muscle softness but there was no correlation with the SE of the muscle block. There was also a low but significant correlation ($P<0.05$, $r = 0.38$) between the IC at 10 mm depth and the SE of the SM muscle block on day 14.

There is a higher correlation between the muscle blocks and SE than between the whole muscle and SE. Perhaps this was due to the fact that the softness of the whole muscle may be more variable. Also the IC at 10 mm depth showed higher correlations than other compression depths and hence would be recommended as the best compression depth to use in experiments.

The SE indicated that the SM was firmer than the LD, conflicting with the IC results which showed that the LD was firmer than the SM. The LD muscles were obviously much softer than the SM, hence it appears that the IC of the LD muscles is not reliable.

The correlation between the IC and SE was surprisingly low for the SM muscles considering that they were firmer, did not collapse under their own weight and hence allowed a more consistent compression than the LD. The correlation of 0.35 ($P<0.05$) between the SE and the whole SM muscle and 0.38 ($P<0.05$) between the SE and the SM muscle blocks were significant but too low for the IC method to be used in parallel with the SE of softness. For comparison, the WBS (Warner, 1928) which is a very common instrumental method used for measuring meat tenderness was shown to explain 80% of the variation in SE (Shackelford *et al.*, 1991), that is, a correlation of $r = 0.9$ with the SE of tenderness. Therefore, as a guide, the correlation between the IC and the SE of softness should ideally be close to this level.

Correlation of Softness Evaluation With pH and Temperature Decline Rates

Table 2.3.5 shows the correlation between muscle softness (SE and Instron compression) with the pH and temperature decline rates (pH K and Temp K) for the LD and SM muscles. There was no correlation ($P>0.05$) between the pH K and the softness of the LD and SM muscles. In contrast, the Temp K was correlated with SE of the whole LD muscle and muscle block for the SM ($P<0.01$, $r = 0.67$, 0.55 respectively) on day 1 which indicated that muscles with faster temperature decline are firmer in texture. Also there was a lower but negative correlation between the Temp K of the SM muscle and the IC on day 1 at 15 and 19 mm depths ($P < 0.05$, $r = -0.37$, -0.39 respectively). The negative correlation indicates that the faster the temperature decline, the softer were the muscles. This is not what we expect to happen and since the IC has

not been giving reliable data for the LD muscle, this conflicting result is probably due to a failure in the compression method. See Chapter 4 for more discussion of the relationship between Temp K and softness.

It is difficult to compare the results of this experiment to that of the literature since there have been no reports which have measured the effects of temperature and pH decline rates on the softness of raw muscles. It is generally known that faster chilling will reduce the incidence of PSE-like occurrence but there have been no reports, which have measured muscle softness and correlated it to the chilling rate. There have been observations that high muscle temperature and rapid pH decline generally result in softer muscles (Locker and Daines, 1975; Tarrant and Mothersill, 1977; Fischer and Hamm, 1980; Honikel *et al.*, 1981), but there are no reports which have measured the softness of raw meat and related it to the temperature and pH decline rates.

2.3.4 Conclusion

The use of a modified IC procedure was successful for measuring raw muscle softness as it detected differences in softness between samples of known texture. In comparing compression depths of 5, 10, 15 and 19 mm, the 10 mm compression depth is the preferred method because it showed higher correlation to the SE and Temp K than the other compression depths. However, the IC showed that the LD was firmer than the SM muscle while the SE showed the opposite. The LD was obviously softer than the SM muscle, hence the SE was more reliable than the IC method. The reason for this conflicting result was that the LD muscles could not be maintained upright and collapsed during the compression, thus compacting on themselves. As a result, the IC suffered from this limitation and hence does not appear to be a suitable method for analysing very soft samples unless they could be prevented from collapsing. Some of the ways of preventing the samples from collapsing could be to put them in containers or cut the samples into smaller standard size and then glue the top end of the pieces to the compression head to prevent the muscles from collapsing. This procedure is discussed and investigated in the next section.

The SE of softness showed differences in softness due to chill rate and to a lesser extent ES than the IC measurements. The SE detected increased softness due to ageing but the IC measurements did not. However, both methods of evaluating softness (IC and SE) did not correlate with the rate of pH decline and hence the suggestion that faster pH decline should result in softer muscles was not observed in this experiment. This could have been because the evaluation of softness was not

very accurate. In contrast, both methods of measuring softness (IC and SE) correlated with temperature decline rate. Therefore the results of this experiment suggest that chilling rate and hence the temperature decline rate had greater effects on softness than the pH decline rate.

2.4 DEVELOPMENT OF INSTRON COMPRESSION TO EVALUATE MUSCLE SOFTNESS CAUSED BY EARLY POST-MORTEM HIGH TEMPERATURE

2.4.1 Introduction

In the previous experiment (2.3), the softness evaluation by IC experienced one major set back. Very soft meat tends to collapse, becomes compact and gives false high compression loads compared to non collapsed samples.. A possible solution of preventing the samples from collapsing would be to cut them into smaller dimensions than 4x5x6 cm and place them in containers. However, from personal observation, restraining samples in a container tends to give higher firmness results than "actual" because the container wall contributes to the firmness by preventing the meat from deforming freely when compressed. If the container is large such that the wall will not interfere, then the samples tend to sag. Therefore using a container to restrain samples does not appear to be a favourable method. An alternative method is to cut the samples into standard size and then attach or fasten one end of each piece to the compression head to prevent them from collapsing. This procedure is similar to that of Pool (1967) who cored cooked meat and glued them to cylindrical disks, but instead of using them for compression they used them in a tensile test. This should eliminate the container wall from affecting the measurement and prevent the samples from collapsing.

In the previous experiment, the LD muscle was compressed in the direction down the length of the muscle (Figure 2.2.1) because it was observed to be much softer than across the width of the LD muscle. It was also hypothesised that the softness of meat would be influenced by its softest direction, that is, parallel to the muscle fibre direction for most muscles, or in the case of the LD muscle, parallel to the length for the muscle. Other researchers have commonly compressed meat perpendicular to the muscle fibres for both the SM and LD muscles to detect the myofibrillar contribution to firmness (Bouton and Harris, 1972; Lepetit *et al.*, 1986; Harris and Shorthose, 1988; Lepetit and Culioli, 1992).

The hypothesis was that if the LD muscles were compressed across the width instead of down the length, this would minimise the samples from collapsing. In addition, the hypotheses were that the orientation of muscle fibres presented to the compression head, and preventing the samples from collapsing using a novel glueing technique, will improve the ability of an instrumental measure of raw meat softness to predict sensory evaluation of meat softness.

Thus the aims of this experiment were to develop new methods of measuring meat softness by 1) coring the samples and gluing them to the compression head thus preventing the samples from collapsing and 2) compressing across the width of LD muscle instead of down the length of the muscle.

2.4.2 Materials and Method

The LD muscle (6.2 ± 0.6 kg) was removed from each side of 38 Hereford beef carcasses from the 3rd to 10th ribs at 20 mins post-mortem. The carcasses had been subjected to ES at 5 min post-slaughter with 10 secs with 200 mA and then for 5 to 10 secs with 180 V during hide removal. The pH and temperature of each loin was measured with a portable pH meter and temperature probe (Hanner Instrument HI9024, USA) and pH probes (Prometary Ltd. Ionode electrode IJ42, Brisbane, Australia) at the anterior, middle and posterior sections. The muscles were individually placed in large plastic bags and kept in an ice container until collection was completed. Five loins were collected on day 1 and then 11 loins on each day for the subsequent 3 days. On each collection day, the loins were transported to the university laboratory and the time lapsed between the first loin being boned to arrival at the university was approximately 1.5 h.

At the university laboratory, the sides were alternately allocated to hot (37 °C) and warm (14 °C) water bath incubation. The pH and temperature of each loin was measured in the three locations given above at 30 min intervals for the first 3.5 h postmortem then at hourly intervals until rigor, defined by pH < 5.8. The loins were transferred to an ice-water bath where the pH and temperature was continued to be measured at 2 h intervals for the next 4 h. The loins were kept in a chiller at 0-2 °C over night.

At 24 h post-mortem, the ultimate pH was measured then the loins were trimmed of all surface fat and connective tissue and chopped in half. Triplicate filter paper wetness was performed (1 at the anterior and 2 at the middle section) on the cut surfaces after 10 mins as described in previous section. After 30 mins bloom, triplicate surface colour (CIE – L*, a*, b*) measurements were made on the same surfaces using a Minolta Chromameter model CR-200, white calibration plate N° 11833161 and D_{65} colour space.

Softness Assessment by Sensory Panel

In both the present study and experiment 2.3 it was observed that the posterior end of the LD muscle was firmer than the anterior end. To eliminate the potential problem of some judges trying to assess the whole length of the LD muscle while others only assessed the anterior end, the LD muscles were cut in half and the softness of the anterior and posterior ends were evaluated as two separate sections. The anterior and posterior ends were laid out as two separate groups in a room at 15 °C. On each day, an extra loin that did not have a matching left and right side was used as a standard in which it was also cut in half and the anterior and posterior ends were then given softness scores by the author. About 10 – 15 untrained judges then 'felt' these standards and used the author's scores as a reference when they assessed the softness of the posterior and anterior ends of each sample. The judges assessed the loins by pressing and squeezing and then scored the softness on a scale of 1 to 8, 1 being extremely soft and 8 extremely firm.

Samples for Laboratory Analysis and Other Measurements

Figure 2.4.1 shows how the LD muscle was cut up then allocated to the different tests using two IC methods (Method A and Method B). After the softness assessment, 2 mm thick cross-sectional slices were cut from the two ends of the posterior and anterior ends. This was to obtain a representative sampling of the whole muscle for the Ca^{2+}-ATPase and protein solubility assays. The posterior section was then weighed (1.35 ± 0.12 Kg) for purge analysis, individually vacuum packaged and kept in a chiller at 2 – 4 °C for 3 days ageing.

From the anterior end of the LD muscle approximately 70, 20, 70 and 250 g samples were allocated to measurements of fat content, sarcomere length, MFI and WBS analyses respectively for day 1 analyses. All the samples except for the sarcomere length samples were individually vacuum packaged and frozen at –20 °C. The sarcomere length samples were frozen but not vacuum packaged. For the drip loss test, rectangular samples from each anterior end of about 6.5 x 4.5 cm, weighing 85 ± 7 g were hung on a piece of wire in inflated plastic bags and kept in a chiller at 2-4 °C for 48 h using a modified method of Honikel *et al.* (1986). The drip loss was the amount of fluid loss expressed as a percentage of the initial sample weight.

On day 3, the samples were removed from vacuum bags, towel dried and re-weighed for purge analysis. Purge loss was the amount of fluid expelled out of the meat expressed as a percentage of the initial sample weight. They were then cut in half and triplicate filter paper wetness

measurements were done. The same group of 10-15 judges from day 1 assessed the softness as previously described. The samples were then cut for MFI and WBS analyses, vacuum packaged and frozen at –20 °C. The remaining portion was cut into 4 cm width along the length of the LD muscle for the Instron compression.

Softness Evaluation by Instron Compression

The remaining anterior portion from day 1 was divided into two blocks of 4 cm width along the length of the LD muscle for the IC using two different methods (Method A and Method B) as shown in Figure 2.3.2 and as described below.

Method A

Figure 2.4.1 shows the IC set up for Method A. It was similar to that described in experiment 2.3, Figure 2.3.1, except in this set up, the compression was across the width of the LD muscle instead of down the direction of its length. As a result, the samples did not collapse out of shape and this eliminated the problem of sagging samples. The sample dimensions were 4 cm thick by 4 cm high. Between 3-4 compression replicates were done on each block. It was obvious that the softness was not homogenous across the samples and hence the compression replicates had to be evenly distributed across the samples to get a representative measurement. Once the samples were compressed with the probe, they were physically damaged and hence could not be re-used for the other compression method.

Method B

Figure 2.4.1 shows the IC setting for Method B. The sample was cored along the length of the LD muscle using a cylindrical corer of 4.5 cm internal diameter to obtain 3-4 cores from the other 4.0 cm meat blocks. One end of each core was paper towel dried before being glued (Araldite epoxy resin, Selleys Australia) onto aluminium disks of 6.5 cm (Figure 2.4.1) diameter which was then screwed onto the IC head. About 6 samples were glued at a time and were left for 3-5 mins to bond to the disks before being mounted on the Instron for compression. The meat core was allowed to hang from the disk while its lower end rested on the compression platform before initiating the compression. All of the cores were 4.0 cm long.

The parameters measured for both methods A and B were the load (force) at different compression depths (5, 8, 11 and 13 mm), the maximum compression depth before reaching a preset load and the total work done by compression to the maximum depth. These compression

depths were used instead of those used in the previous two sections (Sections 2.2 and 2.3) because the compression force at more intervals and at lower depths was required. Experiments 2.2 and 2.3 showed that the lower depths gave more consistent readings and thus were tested in this experiment.

Sarcomere Length

The sarcomere length was measured using a laser diffraction instrument custom-made by the University of New England, Armidale, Australia. From frozen samples, thin slices were shaved off the muscle in the longitudinal direction of the fibre and placed between two microscope slides. A laser beam was passed through the slide and the distance between the diffracted bands measured. The sarcomere length was calculated using the following equation;

Sarcomere Length (μm) = 0.635/sin [arctan d/75] where d = distance (mm) of diffraction bands measured.

Fat content measurement

Fat content measurement was performed according to a modified method of Dransfield (1977). Frozen samples were thawed in cold water, finely chopped and duplicate 30 g samples were freeze dried. The fat content was extracted for 4.0 h in a Soxhlet with 40-60 °C petroleum spirit. The petroleum spirit was removed by a rotary evaporator with vacuum and the remaining fat was further dried in a 70 °C oven for 3-4 h, the content was weighed by difference and the amount of fat was expressed as a percentage of the wet sample weight.

The MFI was performed as described in Chapter 2.1 using 30 sec at low speed setting on the Waring blender.

Statistical Analyses:

The pH decline was modeled using an exponential function $pH(t) = pH\infty + (pH_o - pH\infty)e^{-Kt}$, where pH(t) = pH at time t, pH_o = pH at time 0, $pH\infty$ = pH at time ∞ and K was the rate constant of pH decline. The temperature decline for both the 37 and 14 °C samples was not modeled using the exponential equation since they were maintained constant at those 2 temperatures pre-rigor. The difference in WBS and MFI values between days 1 and 3 were used as indicators of ageing. To test for significant differences, all the parameters were analysed by ANOVA using General Linear Model of SPSS statistical software (version 9) (SPSS Inc. Chicago, Illinois, USA).

2.4.3 Results and Discussion

Temperature and pH declines

The temperature of the muscles in the warm water bath (14 °C) decreased rapidly from 38 °C (carcass temperature) to approximately 18 °C within 6 hours post-mortem while the temperature of muscles in the hot water bath (38 °C) was maintained at 38 °C until rigor (see Figure 3.1 in Chapter 3). Subsequently, the pH decline of the hot samples as measured by the decline rate constant (pH K) was faster ($P<0.05$) than the warm samples. Thus the hot muscles reached rigor much faster than the warm muscles (see Figure 3.2 in Chapter 3).

Sensory evaluation of muscle softness

Table 2.4.1 shows that the SE did not detect differences ($P>0.05$) in softness between the hot and warm treatments in either the anterior or posterior ends of the LD muscle at day 1 or day 3. The lack of difference between treatments could be for one of three reasons, i) the SE method was not sensitive enough to detect differences in softness due to large variation in the softness scores within each sample, or ii) the 2 temperatures had not caused a large difference in softness or iii) more samples were needed to detect a difference due to large variation between the SE scores. Point iii) is most likely as there was a trend for the anterior section of the warm samples to be firmer (higher value) on day 1 ($P = 0.10$) and the posterior end to be softer by day 3 ($P = 0.14$)) compared to the hot samples. Furthermore, the objective compression data showed significant differences to support this as discussed below.

The incubation temperature of 37 °C combined with ES was considered an extreme condition that should have caused significantly softer muscles than the 14 °C. Locker and Daines (1975) described the softness of beef *Sternomandibularis* (STM) muscles entering rigor at 37 °C as "increased tendency of fibre bundles to slide over each other and accurate cutting with a knife became difficult. A cube of meat tended to flop into a rhomboid and the effects were independent to colour change". Hence it was surprising that the SE did not detect any difference in softness between the two temperatures. A study done by Trout (1992) using 6 trained SE panellists could not detect differences in softness between 460 normal and 122 PSE quality pork loins. However, the 6 trained SE panellists did pick up differences in softness between normal and extremely PSE pork loins. According to the results obtained by Trout (1992) and the large number of samples used in his study, it is doubtful that SE of softness could differentiate the softness of beef loins varying between PSE and normal quality.

The posterior end on day 1 was firmer than the anterior end on day 1 and firmer than posterior end on day 3 ($P<0.05$, statistical analysis not presented). This confirmed the initial observation that the posterior end of the LD was firmer than the anterior end and hence the softness of the 2 ends had to be assessed separately to avoid differences in softness due to location within the muscle.

Evaluation of Muscle Softness by Two Instron Compression Methods

Table 2.4.2 shows the softness evaluation by two IC methods. Compression Method A on the anterior end of the LD muscle on day 1 showed that the hot samples were softer ($P<0.05$) than the warm samples at 5 and 8 mm depths and tended to be softer ($P<0.10$) at 11 mm. As the compression depth increased beyond 8 mm, the difference in softness between the hot and warm samples disappeared. This suggests that the difference in softness was greater and more detectable at the muscle surface than deeper into the muscles when they were compressed across the fibres. The other 2 parameters, work done and maximum compression depth did not detect any difference in softness between hot and warm treatments.

In contrast, compression Method B on the anterior end of the LD muscle on day 1 did not detect any difference ($P>0.05$) in softness between the hot and the warm samples. However, Method B did show that the hot samples of the posterior end of the LD muscle were firmer than the warm samples by day 3. Method A was not used on day 3 due to a lack of sample.

The two new IC methods avoided the problems of very soft meat collapsing but have also gave conflicting results. Method A, which compressed across the length of LD muscle showed differences in softness between the hot and the warm samples. Method B, which compressed cored samples along the length of the LD muscle (i.e. close to being parallel to the muscle fibre direction) did not show any difference in softness on day 1 but did on day 3. It has been suggested that compression perpendicular to muscle fibre measures the myofibrillar contribution to softness while compression parallel to the muscle fibre measures the contribution of connective tissues to softness (Bouton and Harris, 1972; Lepetit et al., 1986; Harris and Shorthose, 1988; Lepetit and Culioli, 1992). This observation was further supported by the high correlation (see discussion below) between the SE and Method B but not with Method A.

The pH Decline Rate and the Sarcomere Length

The pH decline rate for the hot samples was much faster than for the warm samples as indicated by higher pH K ($P<0.01$), thus showing that high (37 °C) early post-mortem temperature caused faster pH decline than medium temperature (14 °C). The large difference in pH decline rate has not shown a corresponding difference in the sarcomere length between the hot and the warm samples ($P>0.05$). This would be expected as the temperature treatments would not have induced cold-shortening although it was thought there may have been some heat shortening in the 37 °C treatment.

Comparison and Correlation of Sensory Evaluation With the Instron Compression

Table 2.4.3 shows the Pearson correlation coefficients (r) and respective P values between SE of softness and two IC methods (Method A and Method B) at 5, 8, 11 and 13 mm compression depths. The SE of the LD anterior end on day 1 did not correlate with Method A. On the other hand, the SE was correlated ($P<0.01$) to Method B for both the anterior and posterior ends of the LD muscle despite the fact that both Method B and the SE did not detect differences in softness between the hot and warm samples. The highest correlation ($r = -0.7$, $P <0.01$) was at maximum compression depth on day 3, but, this parameter did not detect differences in softness between the hot and warm samples. In contrast, the compression loads between 5-11 mm depth had slightly lower correlations ($r = 0.55$ to 0.62, $P <0.05$), but could detect differences in softness ($P<0.05$) between the hot and warm samples.

The two IC methods gave conflicting results. Method A showed differences in softness between the hot and warm samples but did not correlate with the SE. On the other hand, Method B was highly correlated with SE but was not able detect differences in softness due to treatments. It is worth re-emphasising that Method A set up allowed greater compression perpendicular to the muscle fibres since it was pressed across the width of the LD rather than down the length as with Method B. The results highlighted the importance of fibre direction when evaluating softness by instrumental compression. Although the judges assessed the softness of the muscles by pressing and squeezing the samples from all directions, it appeared that their overall perception of softness was emphasised down the length of the LD muscle rather than across the width of the muscle that tended to be firmer. Perhaps this may explain why there was a high correlation between the SE and Method B but not with Method A. Previous studies (Bouton and Harris, 1972; Sale *et al.*, 1984) have used instrumental compression to measure meat softness but did not conduct a SE of the softness at the same time to compare whether the two methods correlate.

Correlation of Softness With pH K

Table 2.4.4 shows the Pearson correlation coefficients (r) and respective P values between the pH decline rate constant (pH K) and fat content (%) with the softness assessment by SE and two IC methods (Method A and Method B) at 5, 8, 11 and 13 mm compression depths. The compression Method A on the LD anterior end on day 1 at 5, 8 and 13 mm depth was correlated ($P<0.05$, $r = -0.50$, -0.38 and -0.4 respectively) with the pH K. The negative correlation indicated that the faster the pH decline (that is, the higher the pH K), the softer were the samples. In addition, the long exposure (from slaughter to rigor) to high temperature may have also contributed directly to muscle softening but it is difficult to determine how much each factor (the pH decline rate and the high temperature) contributed to muscle softening. These results support previous reports that higher temperature resulted in faster pH decline and softer samples (Locker and Daines, 1975; Tarrant and Mothersill, 1977; Offer, 1991).

In contrast, the softness results from Method B and the SE (anterior end day 1 and posterior end day 1 & 3) did not correlate ($P>0.05$) with the pH K. This was not surprising since these two methods (Method B and the SE) could not pick up differences in softness between the hot and the warm samples and that these two methods were highly correlated to each other.

Correlation of Muscle Softness With Fat Content

The average fat content of the LD muscles was 1.6% of the wet sample weight which showed that the meat was very lean compared to that of 4.2 % reported by the Meat and Livestock Australia (2001). Dransfield (1977) reported an average value of 5.3% from the LD of two 18 month old grass-fed steers which were a similar age to the cattle in this experiment and Wood (1991) reported that 1 to 7% was a common range for beef LD muscles from grass-fed cattle.

The fat content was correlated to the compression Method A at 11 mm and at maximum compression depth ($P<0.05$, $r = -0.43$, -0.41 respectively) and also with the SE of the posterior end on day 1 ($P<0.05$, $r = 0.45$). Since there was no consistent trend in the correlation between the SE and the fat content, it is difficult to say whether these correlations are reliable.

The softness measurements using Method A were negatively correlated to the fat content which indicated that the higher the compression loads the lower the fat content. That is, firmer muscles have lower fat content. In contrast, the softness measurements using Method B and the SE

showed positive relationship to the fat content which indicated that the higher the fat content, the firmer were the muscles. It seemed that when the muscles were compressed across the fibre, the softness was reduced by higher fat content, while pressing parallel to the length of the LD muscle, the softness increased with increasing fat content. As discussed previously, compression perpendicular to the muscle fibres measures the myofibrillar contribution to firmness while compression parallel to muscle fibres measures only the connective tissues contribution to firmness (Bouton and Harris, 1972; Lepetit *et al.*, 1986; Harris and Shorthose, 1988; Lepetit and Culioli, 1992). Therefore the results indicate that the myofibrillar protein is firmer than the intramuscular fat. In addition, the positive correlation between the SE of softness and the fat content tend to confirm that the judges emphasised their assessment of softness down the length of the LD muscle which is the softer direction, rather than across the width of the muscle. This supports the hypothesis that the IC should be parallel to the length of the LD muscle.

2.4.4 Conclusion

The SE did not detect differences in softness between the hot and warm samples but did show that the posterior was firmer than the anterior section and that the posterior section on day 1 was firmer than day 3. There was no correlation between SE and the pH K and only the SE of the posterior section on day 1 was positively correlated with the fat content. The SE of muscle softness only tended to show that the hot samples were softer than the warm samples on day 1. Perhaps with more samples being assessed and more time spent on training the sensory panel, the results could have been more conclusive.

Both of the IC methods (Method A and B) avoided the problem of the muscle collapsing. However, only the IC that was perpendicular to the muscle fibres (Method A) showed that the hot samples were softer than the warm samples and that faster pH decline and higher fat content caused softer muscles on day 1. The IC of the cored samples parallel to the muscle fibres on day 1 did not show any difference in softness between the hot and warm samples and also did not correlate with the pH K or fat content.

The SE of softness correlated with the IC that was down the length of the LD muscle (Method B) but not with the compression that was across the width of the LD muscle (Method A). This suggests that in SE the softness is emphasised along the muscle fibre direction and not across the

fibre. These conflicting results strongly emphasised the importance of fibre direction when it comes to softness assessment, especially with instrumental methods. Ideally, for an instrumental method to be used to replace a sensory panel, the two methods should be highly correlated. However, a high correlation between two methods that can not detect differences in softness would also be useless. The results indicated that the Instron methods need to be fine tuned to reduce variation, and that the SE panel needs to be well trained in softness assessment to make the method more sensitive in order to detect subtle differences in softness.

2.5 OVERALL CONCLUSION

In Chapter 2.2, it was shown that the IC could detect differences in softness between two groups of extremely soft and normal textured beef SM muscles. The Instron measurements also correlated with the SE results. However, this IC method was not suitable when it was used to measure the softness of raw beef SM and LD muscles which had been ES and chilled at different rates to determine the effects of rapid pH and temperature decline on muscle softness (Chapter 2.3). The LD muscle collapsed under its own weight, causing the meat to be compact and hence resulted in higher compression load reading than if it had not collapsed. This made the LD muscle appear firmer than the SM muscle but it was obvious from SE that the SM muscle was much firmer than the LD. This result casts doubt on the reliability of other softness measurements that were designed to investigate the effects of rapid pH and temperature decline on muscle softness.

In Chapter 2.4 the LD muscle was prevented from collapsing by using two methods, i) gluing the top end of the meat core to a disk that was attached to the IC head and ii) positioning the LD muscle so that the compression was perpendicular to the muscle fibre. Compression of the meat cores was correlated to the SE results but did not detect differences in muscle softness due to treatments. In contrast, compression perpendicular to the muscle fibre (Method A) did not correlate to the SE results but was able to show differences in muscle softness due to temperature treatments and correlated to the pH K and the fat content of the muscle. The compression depths of 5 and 11 mm appeared to be the best parameters to detect differences in softness.

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2.1.1: The effects of homogenisation speed and time and days of ageing (day 1 vs day 14) on the MFI values of beef *Longissimus dorsi* muscles. The means are least square means.

	LOW speed			HIGH speed				P value		
Time (sec)	30	60	90	15	30	60	SED[2]	Speed	Time	SxT
Day 1	41[a]	70[b]	77[c]	34[d]	58[e]	75[c]	3.2	0.001	0.001	0.27
Day 14	81[a]	87[b]	87[b]	80[a]	84[ab]	88[b]	2.8	0.29	0.010	0.66
Difference[1]	40[a]	17[b]	10[b]	46[a]	26[c]	14[b]	4.8	0.01	0.001	0.10

[abcde] Values within rows lacking common superscripts are significantly different ($P<0.05$).
[1] Difference = MFI Day 14 – MFI Day 1
[2] SED = standard error of difference

Table 2.1.2: The effects of homogenisation method (M), the ageing days (D) and the phase (P) of the samples on the MFI values of beef *Longissimus dorsi* muscles. The means are least square means.

	Method		Day		Phases		P value			
	LS30[1]	HS15[2]	1	14	fresh	frozen	SED[3]	M	D	P
MFI	32	27	30	71	59	41	3.4	0.011	0.001	0.001
Difference - (Day 14 – 1)	41	42	NC	NC	43	39	4.4	0.56	NC	0.10

[1] LS30 = Low speed at 30 sec homogenisation
[2] HS15 = Hi speed at 15 sec homogenisation
[3] SED = standard error of difference
NC = not calculate

Table 2.2. 1: Sensory evaluation (SE) of softness and Instron compression (IC) at 3 different depths for 4 *Semimembranosus* (SM) muscles. The number of judges used for the whole SM muscle on day 1, muscle blocks on days 1 and 6 were 16, 16 and 9 respectively.

(n = 16)	Whole muscle day 1				Muscle blocks day 1				Muscle blocks day 6				SED
	Soft		Firm		Soft		Firm		Soft		Firm		
Sample No	I	III	II	IV	I	III	II	IV	I	III	II	IV	
SE score[1]	3.8^{ae}	3.4^{e}	5.2^{cd}	6.1^{f}	2.5^{b}	3.4^{e}	4.5^{ac}	5.3^{cg}	2.0^{b}	2.4^{b}	5.6^{dfg}	6.0^{fg}	0.5
IC Load[2] (Kg) at													
5 mm					0.051^{a}	0.050^{a}	0.10^{a}	0.13^{a}	0.061^{a}	0.039^{a}	0.092^{a}	0.080^{a}	0.05
10 mm					0.32^{a}	0.30^{a}	0.83^{b}	0.82^{b}	***0.66***	0.18^{a}	0.56^{c}	0.27^{a}	0.08
15 mm					2.0^{b}	2.0^{b}	3.1^{c}	3.2^{c}	***6.2***	0.94^{a}	3.1^{c}	1.5^{d}	0.2

Samples I and III were from an old cow, samples II and IV were from normal prime beef.

[1] 1 = Extremely soft, 8 = extremely firm (Trout, 1992).

[2] Each IC value is a mean of 3 replicates.

^{abcdefg} Values lacking common superscript within a row are significantly different ($P<0.05$).

*Sample I of IC on day 6 was excluded from the correlation since its compression values were far too high and thus were treated as outliers.

Table 2.2.2: Correlation matrix showing Pearson's correlation coefficients (r) between Instron compression (IC) measurements and sensory evaluation scores.

Sensory Evaluation	Whole muscle day 1	Muscle blocks day 1	Muscle blocks day 6
Whole muscle day 1	-	0.92	0.97
Muscle blocks day 1	-	-	0.96
IC - day 1 at depths of;			
5 mm	0.99	0.96	-
10 mm	0.96	0.92	-
15 mm	0.97	0.95	-

All correlations were significant at $P<0.01$.

Each correlation value coefficient is estimated using 4 samples and each sample had an average of 3 compressions and 16, 16 and 9 softness scores for Whole muscle on day 1, Muscle block on day 1 and Muscle block on day 6 respectively.

Table 2.3.1: The effects of muscle electrical stimulation (ES) and chilling rate on the softness of whole *Semimembranosus* (SM) and *Longissimus dorsi* (LD) muscles on day 1, the SM and LD muscle block on day 1 and the SM muscle block on day 14 assessed by sensory evaluation.

Sensory Evaluation[10]	muscle	10s ES[1]		40s ES[2]		mean	SED[5]	P values						
		RC[3]	SC[4]	RC	SC			M[6]	S[7]	C[8]	MxE	MxC	ExC	MxExC
Whole muscle Day 1	LD	4.9	3.6	5.2	3.9	4.5	0.05	0.071	0.071	0.001	0.098	0.002	NS[9]	NS
	SM	4.9	4.5	4.8	4.6	4.7[a]								
Muscle block Day 1	LD	3.4	2.9	4.1	2.8	3.3	0.5	0.001	NS	0.001	NS	NS	NS	NS
	SM	5.0	3.4	5.3	3.8	4.4[a]								
Day 14	SM	3.9	3.8	4.0	3.8	3.9[b]	0.4	ND	NS	NS	ND	ND	NS	ND

[1]10s ES = 10 secs electrical stimulation (45 V & 500 mA), [2]40s ES = 40 secs electrical stimulation (45 V & 500 mA)

[3]RC = Rapid Chill (1 °C for 10 h), [4]SC = Slow Chill (18 °C for 2 h and then at 6 °C for 8 h)

[5]SED = standard error of difference.

[6]M = muscle, [7]E = electrical stimulation, [8]C = chill rate,

[9]NS = not significant, P>0.10

[10] softness score: 1 = extremely soft, 8 = extremely firm

[ab] Values lacking in common superscript within the column are significantly different at P<0.01, SED = 0.21

ND = not determined

Table 2.3.2: The effects of muscle electrical stimulation (ES) and chilling rate on the softness of *Semimembranosus* and *Longissimus dorsi* muscle block on day 1 as measured by the Instron compression (IC) load (Kg) at 5, 10, 15 and 19 mm compression depths.

Instron Compression	muscle	10s ES[1]		40s ES[2]		mean	SED[5]	P values						
		RC[3]	SC[4]	RC	SC			M[6]	S[7]	C[8]	MxE	MxC	ExC	MxExC
Depth (mm)	LD	0.11	0.11	0.12	0.11	0.11	0.02	0.001	NS[13]	NS	NS	NS	NS	NS
5	SM	0.08	0.09	0.06	0.07	0.07								
10	LD	0.55	0.39	0.60	0.47	0.50	0.09	0.001	NS	NS	NS	0.040	NS	NS
	SM	0.21	0.22	0.21	0.27	0.23								
15	LD	1.62	1.62	1.81	1.37	1.60	0.31	0.001	NS	NS	NS	NS	NS	NS
	SM	0.88	0.98	0.81	1.12	0.94								
19	LD	2.77	2.96	2.94	2.67	2.83	0.52	0.071	NS	NS	NS	NS	NS	NS
	SM	2.06	2.54	2.00	2.93	2.38								

[1]10s ES = 10 secs electrical stimulation (45 V & 500 mA), [2]40s ES = 40 secs electrical stimulation (45 V & 500 mA)

[3]RC = Rapid Chill (1 °C for 10 h), [4]SC = Slow Chill (18 °C for 2 h and then at 6 °C for 8 h)

[5]SED = standard error of difference, [6]M = muscle, [7]E = electrical stimulation, [8]C = chill rate, [9]NS = not significant, P>0.10

Table 2.3.3: The effects of electrical stimulation (ES) and chill rate on the softness of *Semimembranosus* muscle block on day 14 as measured by the Instron compression (IC) loads (Kg) at 5, 10, 15 and 19 mm compression depths.

Instron	10s ES[1]		40s ES[2]		P values			
Compression	RC[3]	SC[4]	RC	SC	SED[5]	E[6]	C[7]	CxE
5	0.18	0.08	0.08	0.09	0.07	NS	NS	NS
10	0.24	0.24	0.23	0.26	0.04	NS	NS	NS
15	0.97	0.87	0.85	0.96	0.18	NS	NS	NS
19	2.79	2.18	2.57	2.97	0.57	NS	NS	NS

[1]10s ES = 10 secs electrical stimulation (45 V & 500 mA),

[2]40s ES = 40 secs electrical stimulation (45 V & 500 mA)

[3]RC = Rapid Chill (1 °C for 10 h),

[4]SC = Slow Chill (18 °C for 2 h and then at 6 °C for 8 h)

[5]SED = standard error of difference,

[6]E = stimulation, [7]C = chill rate, [8]NS = not significant, P>0.10

Table 2.3.4: Pearson correlation coefficients (r) and their respective P values between the Instron compression (IC) loads at 5, 10, 15 and 19 mm depths and the sensory evaluation (SE) of softness of the whole *Semimembranosus* (SM) and *Longissimus dorsi* (LD) muscles on day 1, the SM and LD muscle blocks on day 1 and the SM muscle blocks on day 14 samples.

		SE Whole muscle, day 1				SE: Muscle block, day 1				SE: Muscle block Day 14, SM	
		LD		SM		LD		SM			
		r	**P**	**r**	**P**	**r**	**P**	**r**	**P**	**r**	**P**
IC- 5 mm	Day 1	0.20	0.26	0.03	0.85	**0.40**	**0.05**	-0.21	0.32	0.04	0.82
	Day 14			0.20	0.22			-0.06	0.75	-0.03	0.84
IC – 10 mm	Day 1	**0.50**	**0.01**	0.29	0.07	**0.79**	**0.01**	-0.33	0.11	-0.04	0.83
	Day 14			0.35	0.13			-0.22	0.31	**0.38**	**0.02**
IC – 15 mm	Day 1	0.28	0.10	**0.35**	**0.03**	**0.75**	**0.01**	-0.25	0.24	0.08	0.62
	Day 14			0.19	0.24			-0.01	0.98	0.25	0.13
IC – 19 mm	Day 1	0.19	0.28	0.30	0.06	**0.65**	**0.01**	-0.33	0.12	0.07	0.69
	Day 14			0.20	0.27			0.05	0.82	0.11	0.53

Each correlation value coefficient is estimated using 40 samples and each sample had an average of 3 compressions and 10 individual judge scores.

Table 2.3.5: Pearson correlation coefficients (r) and the respective P values between the pH and temperature decline rate constants (pH K and Temp K respectively) with the Instron compression (IC) loads at 5, 10, 15 and 19 mm depths and the sensory evaluation (SE) of softness of the whole *Semimembranosus* (SM) and *Longissimus dorsi* (LD) muscle on day 1, the SM and *LD* muscle block on day 1 and the SM muscle block on day 14.

Softness Evaluation		**pH K**				**Temp K**			
		LD		SM		LD		SM	
		r	P	r	P	r	P	r	P
IC- 5 mm	Day 1	-0.02	0.99	-0.06	0.70	0.07	0.72	-0.05	0.78
	Day 14			-0.11	0.51			-0.03	0.87
IC – 10 mm	Day 1	0.02	0.99	0.27	0.10	0.25	0.18	-0.28	0.09
	Day 14			0.09	0.58			0.03	0.84
IC – 15 mm	Day 1	-0.04	0.81	0.23	0.16	0.10	0.58	**-0.37**	**0.02**
	Day 14			-0.11	0.50			0.28	0.09
IC – 19 mm	Day 1	-0.03	0.86	0.24	0.14	-0.04	0.98	**-0.39**	**0.01**
	Day 14			0.10	0.61			0.22	0.23
SE: Whole muscle, Day 1		0.14	0.39	-0.05	0.75	**0.67**	**0.001**	-0.18	0.27
SE: Muscle block, Day 1		-0.02	0.93	0.13	0.56	0.35	0.12	**0.55**	**0.005**
SE: Muscle block, Day 14				-0.02	0.92			0.07	0.69

Table 2.4.1: The effects of early postmortem temperature control (37 vs 14 °C) on muscle softness (as measured by sensory evaluation and Instron compression (IC)), pH decline rate (pH K) and sarcomere lengths on beef *Longissimus dorsi* muscles after 1 or 3 days ageing.

Variable	Hot (37 °C)	Warm (14 °C)	SED	P
Sensory evaluation*				
Anterior End day 1	3.71	4.36	0.38	0.10
Posterior End day 1	5.10	5.22	0.45	0.79
Posterior End day 3	4.41	3.88	0.35	0.14
IC Method A: Anterior End Day 1				
Load at - 5 mm depth (Kg)	0.27	0.35	0.03	0.02
- 8 mm	0.96	1.28	0.13	0.02
- 11 mm	2.20	2.62	0.24	0.09
- 13 mm	2.59	2.98	0.29	0.19
Work done (J)	18.4	17.6	0.93	0.41
Max compression depth (mm)	15.3	14.4	0.61	0.13
IC Method B: Anterior End Day 1				
Load at - 5 mm depth (g)	10.3	10.9	0.9	0.55
- 8 mm	17.0	18.3	2.7	0.63
- 11 mm	35.7	35.9	10	0.99
- 13 mm	75.2	71.2	26	0.88
Work done (J)	1.70	1.69	0.01	0.81
Max compression depth (mm)	3.99	3.83	0.21	0.45
IC Method B: Posterior End Day 3				
Load at - 5 mm depth (g)	19.4	12.5	1.9	0.01
- 8 mm	41.6	21.0	6.4	0.01
- 11 mm	120	55.6	27	0.02
- 13 mm	251	125	62	0.05
Work done (J)	1.67	1.66	0.02	0.64
Max compression depth (mm)	4.33	3.73	0.34	0.09
Potential Factors That Can Affect Softness				
pH K (pH unit/h)	0.37	0.22	0.036	0.001
Sarcomere Length (μm)	1.70	1.72	0.056	0.59

*scoring scale, 1 = extremely soft, 8 = extremely firm

Table 2.4.2: Pearson correlation coefficients (r) and respective P values between sensory evaluation of softness and two Instron compression (IC) methods (Method A and Method B) at 5, 8, 11 and 13 mm compression depths.

Instron Compression	Sensory Evaluation Day 1: Anterior End	
IC Method A: Anterior End, Day 1	r	P
Load at - 5 mm depth	0.12	0.53
- 8 mm	-0.04	0.82
- 11 mm	-0.03	0.89
- 13 mm	-0.13	0.50
Work done	.045	0.81
Max compression depth	0.06	0.75
IC Method B: Anterior End, Day 1		
Load at - 5 mm depth	0.63	0.01
- 8 mm	0.63	0.01
- 11 mm	0.57	0.01
- 13 mm	0.59	0.01
Work done	0.33	0.08
Max compression depth	-0.68	0.01
IC Method B: Posterior End, Day 3	Sensory Evaluation Day 3: Posterior End	
Load at - 5 mm depth	0.55	0.02
- 8 mm	0.59	0.01
- 11 mm	0.62	0.01
- 13 mm	0.63	0.01
Work done	0.09	0.65
Max compression depth	-0.70	0.01

Table 2.4.3: Pearson correlation coefficients (r) and respective P values between the pH decline rate constant (pH K) and fat content (%) with the softness assessment by sensory evaluation and two Instron compression (IC) methods (Method A and Method B) at 5, 8, 11 and 13 mm compression depths after 1 or 3 days ageing.

Correlation Matrix	pH K		Fat Content	
IC Method A: Anterior End, Day 1	r	P	r	P
Load at - 5 mm depth	-0.50	0.01	-0.32	0.08
- 8 mm	-0.38	0.04	-0.35	0.06
- 11 mm	-0.29	0.12	-0.43	0.02
- 13 mm	-0.40	0.03	-0.32	0.08
Work done	0.15	0.44	0.18	0.35
Max compression depth	0.33	0.07	-0.41	0.02
IC Method B: Anterior End, Day 1				
Load at - 5 mm depth	-0.25	0.16	0.21	0.22
- 8 mm	-0.28	0.11	0.12	0.48
- 11 mm	-0.20	0.27	0.12	0.51
- 13 mm	-0.16	0.41	0.10	0.61
Work done	0.20	0.29	0.10	0.60
Max compression depth	0.17	0.38	-0.29	0.12
IC Method B: Posterior End, Day 3				
Load at - 5 mm depth	0.34	0.07	0.20	0.30
- 8 mm	0.41	0.02	0.23	0.22
- 11 mm	0.36	0.05	0.21	0.28
- 13 mm	0.27	0.14	0.18	0.33
Work done	0.55	0.01	0.20	0.28
Max compression depth	-0.07	0.70	-0.15	0.43
Sensory evaluation				
Anterior End day 1	-0.24	0.18	0.24	0.16
Posterior End day 1	0.04	0.83	0.45	0.01
Posterior End day 3	0.09	0.64	0.34	0.07

Figure 2.2.1: Diagram showing the set up for the Instron compression of the *Semimembranosus* muscle blocks using a cylindrical probe.

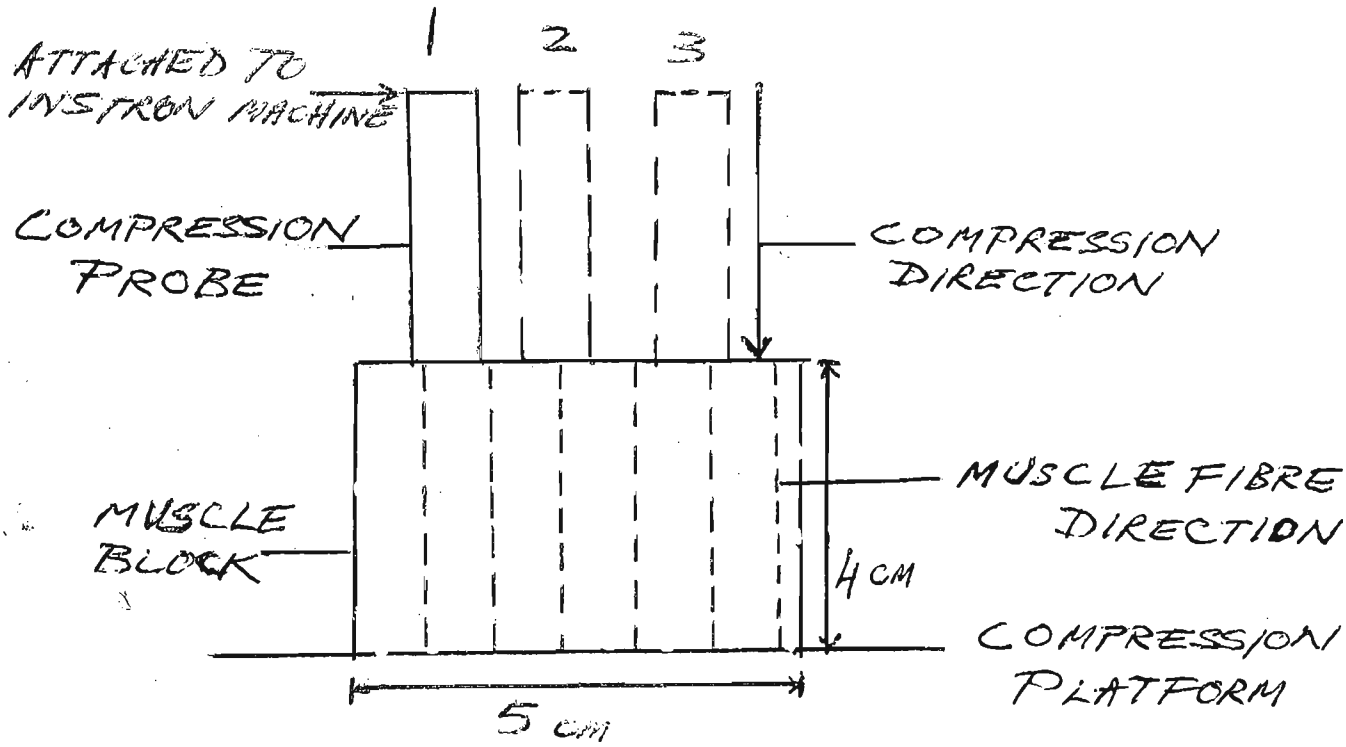


Figure 2.3.1: Diagram showing the set up for the Instron compression of the *Longissimus dorsi* muscle blocks using a cylindrical probe.

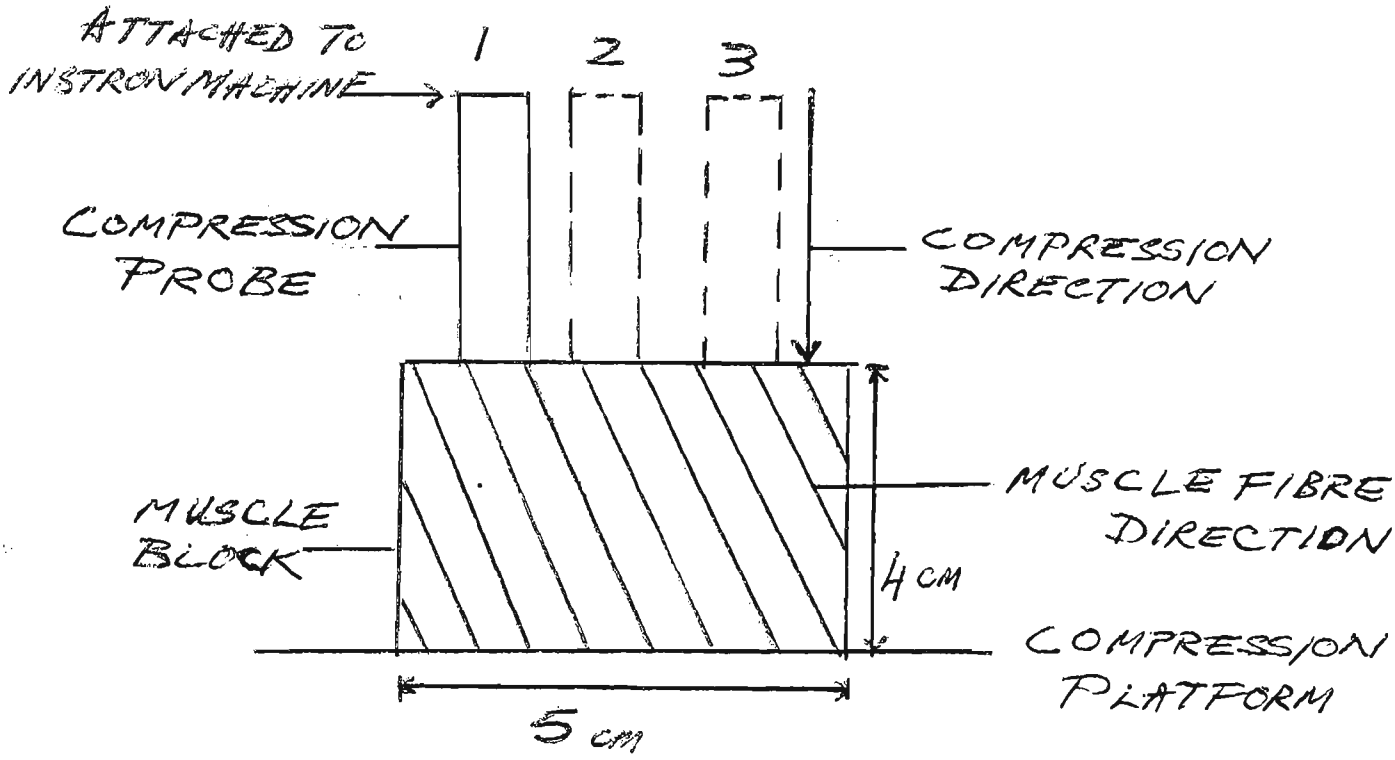
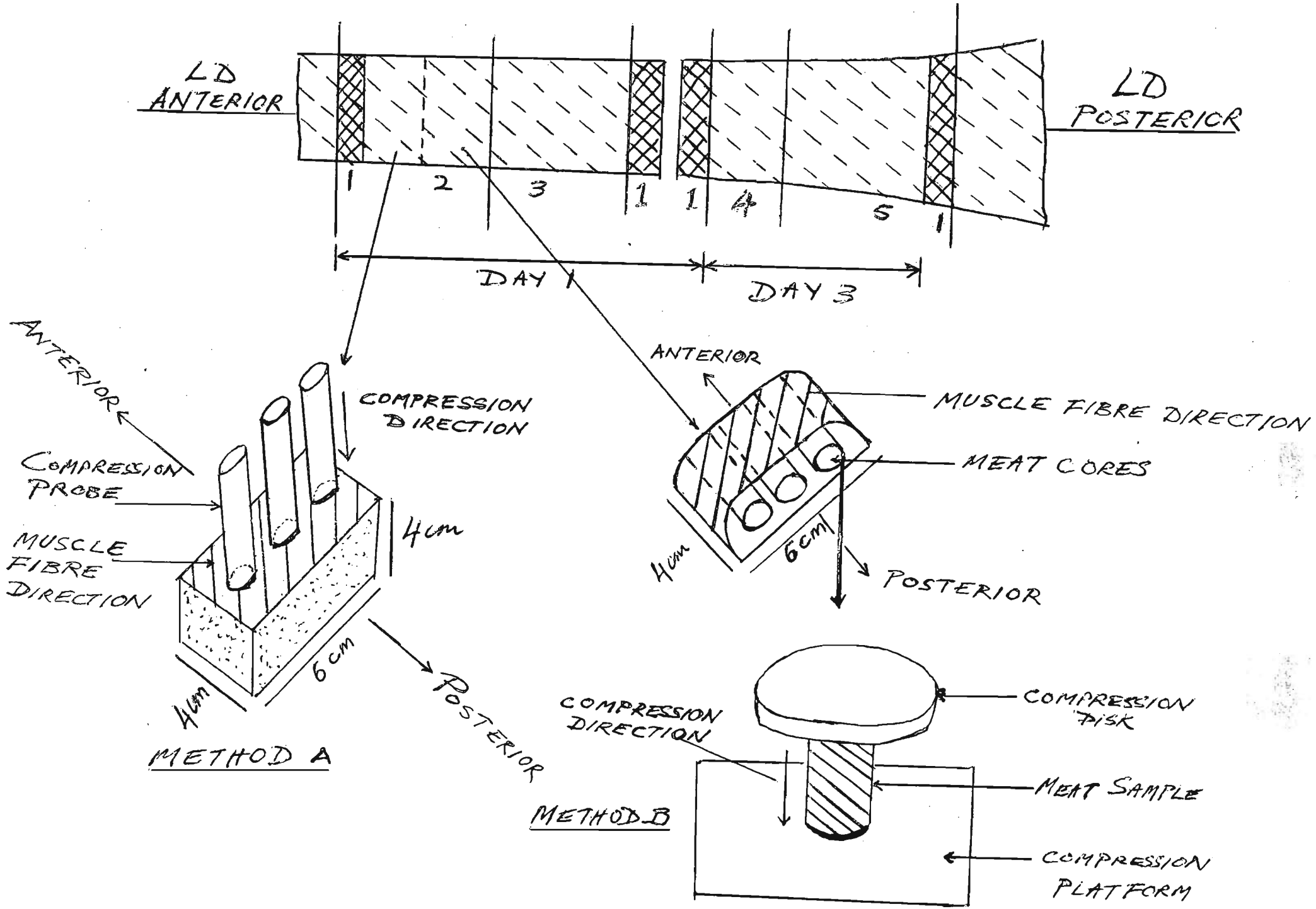


Figure 2.4.1 (next page): Sample allocation from the *Longissimus dorsi* muscle for laboratory analyses and softness assessment by the sensory evaluation and Instron compression (Method A and Method B) for days 1 and 3.

Sample allocation for different analyses (from the diagram)

Section	Analysis
1	Colour, filter paper, myofibrillar ATPase, protein solubility
2	Sensory evaluation, Instron compression
3	WBS, MFI, fat content, sarcomere length
4	Sensory evaluation, Instron compression
5	WBS, MFI


LD ANTERIOR
LD POSTERIOR
1
2
3
1
1
4
5
1
DAY 1
DAY 3
ANTERIOR
COMPRESSION DIRECTION
COMPRESSION PROBE
MUSCLE FIBRE DIRECTION
4cm
4cm
6cm
POSTERIOR
METHOD A
ANTERIOR
MUSCLE FIBRE DIRECTION
MEAT CORES
4cm
6cm
POSTERIOR
COMPRESSION DISK
COMPRESSION DIRECTION
MEAT SAMPLE
METHOD B
COMPRESSION PLATFORM

CHAPTER 3

THE EFFECTS OF EARLY POST-MORTEM HIGH TEMPERATURE ON MUSCLE SOFTNESS AND WATER HOLDING CAPACITY OF BEEF

3.1 Introduction

Fresh meat has important quality characteristics such as tenderness, juiciness, colour and texture that can affect its attractiveness to the consumer and hence it's marketability. A major quality problem in pork is a condition called PSE, which is when the muscle is a paler, softer and loses fluid (exudate) excessively. This condition also exists in beef but to a less severe degree. Both the meat industry and researchers recognise that PSE in beef as a problem (Locker and Daines, 1975; Fischer and Hamm, 1980; Offer, 1991) but there is limited research.

Many researchers have suggested that PSE in pork is caused by both rapid pH decline and high muscle temperature which lead to protein denaturation and subsequently poor WHC of the muscles (Sayre and Briskey, 1963; Penny, 1977; Tarrant and Mothersill, 1977; Fischer and Hamm, 1980; Bruce and Ball, 1990). Furthermore, it is not only the rapid pH decline and high muscle temperature but also the duration of time the muscles are exposed to high temperature that also contribute to protein denaturation (Penny, 1967; Monin and Laborde, 1985; Offer, 1991). Sayre and Briskey (1963) found that muscles which entered rigor at a high temperature resulted in lower sarcoplasmic protein solubility even though their ultimate pH were higher than samples that entered rigor at a lower temperature.

Tarrant and Mothersill (1977) found in 3 bovine muscles (*Adductor* (AD), ST and BF), that at 8 cm depth, the pH decline was faster and the temperature remained higher for a longer period than at 1.5 cm. A high temperature of 30 °C had lasted for 3 h while the pH values of less than 6.0 were obtained at the 8 cm depth. Consequently, the samples measured at 8 cm depth had greater drip loss, myofibrillar denaturation and were paler, softer and wetter than at 1.5 cm depth when examined at 2 days post-mortem. These reports highlight the effects of rapid pH decline and the

duration of high temperature on protein denaturation and subsequently on the WHC and muscle softness. If these observations are true, then excessive muscle softness can be induced by generating rapid pH decline and prolonged exposure to high temperature early post-mortem.

During post-mortem tenderisation of meat, there are breaks within the myofibrils beside the Z-disks, degradation of the intermyofibril linkages (desmin) (Uytterhaegen *et al.*, 1994; Taylor *et al.*, 1995) and the structural proteins such as filamin and nebulin (Dransfield, 1994; Alarcon-Rojo and Dransfield, 1995; Koohmaraie, 1996). Taylor *et al.* (1995) reported that the costameres (γ-actin, vinculin, β-spectrin, talin) which link the cell membrane to the myofibrils are degraded by about 72 h post-mortem. Therefore, the separation of cell membrane in its linkages from the myofibrils would allow fluid to leave the muscle cell and hence contribute to the exudate.

It would be expected that fluid loss would increase over time post-slaughter as the muscle structure becomes more disintegrated due to proteolysis since the bulk of the water is held in the muscle by the myofibrils. Boakye and Mittal (1993) found that pressed juice from porcine LD muscle increased by 4% from 54.7 on day 0 to 58.5% of expressible fluid by 12 days post-mortem. A similar trend has been found with reddish-pink, firm and normal (RFN) quality pork in which the initial amount of drip was low and increased to a maximum by 4 days post-mortem storage (van Laack and Smulders, 1992). However, if proteolysis is accelerated post rigor, then fluid loss similar to that of PSE meat could be accelerated compared to that of normal muscles. With PSE muscles, it is commonly observed that softer samples are usually consistent with poorer WHC. Some studies have shown that muscles entering rigor at a high temperature (e.g. 35°C) tenderise less during ageing than muscles entering rigor at warm temperature (e.g. 15°C) (Locker and Daines, 1975; Devine *et al.*, 1999). Conflicting with this are reports that PSE-like muscles have a higher breakdown of nebulin and titin at 24 h post-mortem and higher protease activity than normal muscles (Whipple *et al.*, 1990b; Ferguson *et al.*, 2000; Hwang *et al.*, 2001).

The amount of intramuscular fat may also affect the softness of raw meat. The fat reduces the amount of myofibrillar protein per unit volume and hence the muscle density. That is, the fibre bundles would have a less rigid structure as the fat content increases (Wood, 1991).

The hypotheses of this experiment are;

1) soft texture in beef muscles is mainly due to insufficient control of the pH and temperature decline.
2) accelerated proteolysis may result in early loss of WHC and hence lead to early development of softer muscles. If there is accelerated proteolysis, then it should be possible for muscles to become softer and have poor WHC without excessive protein denaturation.
3) increasing the fat content of muscles will lead to softer the texture since fat is softer than myofibrillar proteins.

The aims of this research were i) to investigate the effects of early post-mortem high temperature and rapid pH decline on the WHC and softness of beef muscles and ii) to investigate the role of protein denaturation, accelerated proteolysis and muscle fat content in determining muscle softness.

3.2 Materials and Method

Please refer to Chapter 2.2.3 as this experiment was conducted at the same time and therefore used the same animals, materials, experimental design and physical measurement methods etc.

Filter Paper Exudate

Surface exudate was measured in triplicate at 4 °C using a 5.5 cm diameter filter paper (Schleicher & Schnell SS589/3, Germany) 5 min after making a cut surface using the method of Kauffman *et al.* (1986) as modified by Trout (1992). The filter papers were placed at the center of three different surfaces, lightly pressed to ensure full contact and removed after 2-3 secs. The wet papers were put back into pre-

weighed plastic tubes, capped and weights recorded 3 h later. The exudate was expressed as mg/cm^2.

Sarcoplasmic Protein Solubility

Both the sarcoplasmic and total protein solubility assays were conducted according to the method of Warner *et al.* (1997) with some modifications as follows. Approximately 20 g of fresh muscle sample, free of fat and connective tissue was finely chopped. Duplicate 2.0 g samples were added to 20 mL of cold phosphate buffer (40 mM sodium phosphate, 1 mM NaN_3, 1 mM EGTA, pH 6.5) and homogenised with an Ultra-Turrax T25 (Janke & Kunkel, Germany) for two 8.0 secs bursts at maximum speed while keeping the samples cold in ice-water. The solution was filtered through a 12.5cm diameter N°1 Whatman filter paper for 1.0 hour. The protein concentration of the filtrate was determined in duplicate by mixing 4.0 mL of biuret reagent (36 mM $CuSO_4$, 3% (v/w) NaOH, 127 mM sodium tartate) to 1.0 mL of the filtrate and reading the absorbance at 540 nm after half an hour (Gornall *et al.*, 1949). BSA (Sigma, Fraction V, A-6793, St. Louis) in the range of 0 – 5.0 mg/mL was used as the standard. A correction blank for back ground absorbance was included by mixing 4.0 mL of a solution containing 127 mM sodium tartate and 3% (v/w) NaOH to 1.0 mL of the filtrate and measuring the absorbance at 540 nm shortly after mixing. This was to correct for absorbances from highly coloured proteins such as haemoglobin that could increase the background absorbance. The sarcoplasmic protein solubility was expressed as mg/g meat.

Ca^{2+}-Activated Myofibrillar ATPase

The Ca^{2+}-activated myofibrillar ATPase method used was a modification from (Warner *et al.*, 1997). Two g of fresh finely chopped sample was added to 20 mL of rigor buffer (RB) (75 mM KCl, 20 mM imidazole, 2.0 mM $MgCl_2$, 2.0 mM EGTA, 2.0 mM NaN_3, pH 7.2) and homogenised as described in the sarcoplasmic protein solubility procedures. The homogenate was centrifuged at 1000 g for 15 min at 4 °C and the supernatant discarded. The homogenisation and centrifugation steps were repeated 2 more times with 20 mL of RB each. After the last homogenisation step, the mixture was filtered through a polyethylene strainer (mesh size 8). The strainer and tube were rinsed with 20 mL RB containing 1% Triton X-100 and combined with

the 20 mL myofibril suspension, the final concentration of Triton X-100 was 0.5%. The solution was mixed, centrifuged, supernatant discarded and the pellet washed once more with 20 mL 0.5% Triton X-100. The pellet was finally washed with 20 mL RB (no Triton X-100) and finely suspended by shaking in 20 mL RB containing 80% glycerol (w/w) and 1.0 mM dithiothreitol (DTT) to be stored at –20 °C for future Ca-activated ATPase analysis.

One mL of the myofibrils stored in 80% glycerol-RB was washed, centrifuged and re-suspended with RB containing 1.0 mM DTT and 1.0 mg/mL BSA and this was repeated 3 times. The protein concentration of the myofibrillar suspension was determined by the biuret method as described above. The RB used to suspend the myofibrils also contained BSA and hence was used as a blank. The myofibrillar suspension was diluted to 0.1 mg/mL with RB containing 1.0 mM $CaCl_2$ but no EGTA. Four 200 μL aliquots were placed into microfuge tubes and kept on ice. 20 μL of 25% trichloroacetic acid (TCA) was added to one of the tubes to denature the proteins to use as a blank.

The ATPase reaction was activated by adding 5.0 μL of 0.2 M ATP per 200 μL of myofibrils suspension while being incubated at 38 °C. A pre-incubation time of 30 secs was used before initiating the reaction. The reaction was stopped after 5.0 min by adding 20 μL of 25% TCA and then placed on ice. Five μL 0.2 M ATP was added to the blank (the denatured myofibrils) after 10 min while being kept on ice. The quantity of the inorganic phosphate liberated was measured later using the method of Carter and Karl (1982). The samples were centrifuged and 100 μL of the supernatant was added to tubes containing 0.35 mL solution A (4 volume 2 N HCl: 3 volume 0.1 M Na_4MoO_4) and 0.40 mL of distilled water. Immediately 0.15 mL of solution B (0.042% (w/v) Malachite Green dye solution in 1% (w/v) polyvinyl alcohol) was mixed in and the mixture was allowed to stand for 2.0 mins. One mL of 7.8% (v/v) H_2SO_4 was then mixed in thoroughly and the absorbance measured at 630 nm after 1 h (Carter and Karl, 1982). Phosphate standards in the concentration range 0 – 60 μM per 0.50 mL were prepared from a phosphorus standard (645 nmoles/mL, Sigma 661-9). The Ca^{2+}-activated myofibrillar ATPase was calculated as μmol Pi/mg protein/min.

Warner-Bratzler Peak Shear Force

The Warner-Bratzler peak shear force was performed according to the method of Bouton and Harris (1972). Meat blocks of 248 ± 16 g were cooked in an 80 °C water bath for 1 hour, immediately cooled under cold running tap water and then kept overnight in a chiller. The following day, the cooked samples were paper-towel dried and weighed for cook loss measurements before cutting into small pieces of 0.67 x 1.5 cm with the larger dimension parallel to the muscle fibres. Each sample had about 10 replicate shears across the muscle fibres with a flat blade of 0.61mm thickness in a similar set up to that described by Bouton and Harris (1972).

3.3 Results and Discussion

pH decline, colour, sarcomere length and fat content measurements.

Table 3.1 shows the effects of early post-mortem temperature (37 vs 14 °C) on the various biochemical analyses and physical measurements of beef LD muscles. Figures 3.1 and 3.2 show the temperature and pH decline curves of the high and medium temperature treatment (37 vs 14 °C) of the LD muscles with time post-slaughter. The pH decline rate constant K was much higher for the hot than the warm samples, thus indicating a much faster (almost double) pH decline rate ($P<0.01$) as would be expected. This result agrees with previous studies that chilling temperatures have significant effects on the rate of pH decline (Penny, 1977; Honikel *et al.*, 1981; Babiker and Lawrie, 1983; Offer, 1991). The muscle lightness (L*), redness (a*) and (b*) measurements also reflected this trend with the hot samples being significantly higher thus lighter and more red and yellow than the warm samples ($P=0.01$ for all). A previous study by Bruce and Ball (1990) also showed that holding beef *Semitendenosus* muscle at 31 °C caused higher L*, a* and b* values than those muscles held at 20 °C. There was a significant correlation ($P<0.05$, $r = 0.36$) between the pH K and a* (Table 3.2).

There was no difference ($P>0.05$) in sarcomere length between the hot and warm samples. The mean values of 1.70 and 1.73 μm for the hot and warm samples respectively appear to be shorter than the reported resting range of 1.89 - 2.0 μm for beef (Cross *et al.*, 1981; Honikel *et al.*, 1986; Judge *et al.*, 1989; Lawrie, 1991).

Honikel *et al.* (1986) found that bovine ST muscle that entered rigor at 38 °C and pH 5.8 to 5.6 had sarcomere shortening of 40 - 50% compared to the normal length of 1.9 - 2.0 μm. Therefore, the sarcomere length of the hot samples in this experiment were expected to be shorter than 1.70 μm since they entered rigor at similar temperature and pH ranges (37 °C and pH 5.9 to 5.7). However, minimum sarcomere shortening was expected from the warm samples since 14 °C is the optimum temperature for muscles to enter rigor (Honikel *et al.*, 1986). The range of 1.70 - 1.73 μm corresponded to 8 - 9% shortening if compared to the normal length of 1.9 - 2.0 μm and this agreed with the value obtained by Honikel *et al.* (1986) for the ST muscle that entered rigor at 14 °C. In keeping with a lack of treatment effect there was no correlation ($P>0.05$, Table 3.2) between the sarcomere length and the pH K.

Protein denaturation and the water holding capacity measurements.

The protein denaturation and WHC measurements (drip loss, filter paper wetness, cook loss) showed consistent and expected effects of early post-mortem high temperature (hot 37 °C) compared to medium temperature (warm 14 °C) on the LD muscles.

The myofibrillar Ca^{2+}-ATPase activity of the hot samples was much lower ($P<0.01$) than the warm samples, hence indicating greater denaturation of the myosin head. Den Hertog-Meischke *et al.* (1997) reported similar myofibrillar ATPase values of 0.30 and 0.32 (μmol Pi/mg protein/min) for ES and non-stimulated beef LD muscles respectively. However, Tarrant and Mothersill (1977) reported a much higher value of 0.54 (μmol Pi/mg protein/min) for non-stimulated beef AD muscle measured at 8 cm depth. The higher value reported by Tarrant and Mothersill (1977) could be due to the difference in the muscle or the analytical method used.

There were significant correlations between the ATPase activity and the pH K, the L* and the a* values ($P<0.01$, $r = -0.49$, -0.49 and -0.63 respectively, Table 3.2). There was also a significant correlation ($P<0.01$, $r = 0.52$) between the ATPase activity and sarcoplasmic protein solubility.

The drip loss, cook loss at days 1 and 3 and filter paper wetness at days 1 and 3 were all higher ($P<0.05$) for the hot samples than for the warm samples. The mean drip loss for each treatment of 1.35 and 1.08% (hot and warm samples respectively) was low compared to that reported by Bruce and Ball (1990) of 2.33 and 0.84% for beef ST muscle kept at moderately high temperature (31 °C for 4 h) and medium temperature (20 °C for 6 h) respectively. Although the muscles were hot boned in this experiment and that of Bruce and Ball (1990), the two experiments used different muscles and hence comparison of drip loss may not be appropriate. Den Hertog-Meischke *et al.* (1997) reported a drip loss of 1.9 and 1.6% for ES and non-stimulated LD muscles but did not find differences between the treatments.

The cook losses for day 1 were 21.0 and 23.4 % for the warm and hot samples respectively (Table 3.1). The results are comparable to that of Locker and Daines (1975) who found that the mean cook loss for unrestrained beef STM muscle held for 7 h at 15 and 37 °C and then kept at 2 °C for 30 h were 26.0 and 30.1% respectively. Figure 3.3 shows that the cook loss increased uniformly by 5.7 and 6.0% between 1 and 3 days post-slaughter for the two temperature treatments. Figure 3.4 shows that the filter paper wetness for the hot samples was higher than for warm samples for both days 1 and 3 but the difference was much smaller on day 3. This was because the warm samples drip loss increased between days 1 and 3 whereas there was no increase for the hot samples. The results indicate that fluid loss from samples is slower when muscle temperature decline is slower due to better chilling. These results suggested that early post-mortem high temperature combined with rapid pH decline not only caused poor WHC but also early development of poor WHC.

Joo *et al.* (1995) and Kim *et al.* (1993) found that the 24 h drip loss from PSE pork LD muscle was much higher than that at the 96 h. Other reports (van Laack and Smulders, 1992; Kim *et al.*, 1993) have shown that normal pork muscles had lower drip loss initially than PSE pork and drip loss then increased with ageing. The trend observed from the filter paper wetness measurements between the hot and the warm samples in this experiment agrees with these reports. However, Warner (1994) found that the drip loss from PSE pork had a rapid increase within the first 24 h and continued to increase slowly to day 9, instead of decreasing. Warner suggested that

the difference was due to experimental procedures, in this case the samples used for drip loss were removed from carcasses 60 mins post-mortem and suspended for the allocated length of time. However, authors who found decreasing drip loss from PSE samples only removed the muscles 24 h post-mortem and at each time period following, rather than leaving samples to remain in continuous suspension throughout the measured times. As a result, the total amount of drip loss is cumulative and if it was not measured from the beginning then it would appear that the amount of drip loss decreases over time since much was lost at the beginning.

Table 3.2 shows the Pearson correlation coefficients (r) between various biochemical and physical measurements of beef LD muscles to demonstrate their relationships. The correlations show that the faster the pH decline, the higher the myosin head denaturation (correlation of pH K and ATPase, $r = -0.49$, $P<0.01$) and the lower the WHC of the muscles as measured by the filter paper wetness on day 1 ($r = 0.38$, $P<0.05$) and cook loss on day 1 ($r = 0.41$, $P<0.05$). Increasing pH decline rate also decreases the redness in the muscles (correlation of pH K and a*, $r = 0.36$, $P<0.05$). The results showed that reduced redness, more protein denaturation and lower WHC could be induced by higher temperature and faster pH decline.

Proteolysis

The WBS for days 1 and 3 and the difference in WBS between day 1 and 3 was not different between the hot and warm samples ($P>0.05$, Table 3.1). The WBS values for the 2 different temperatures (37 and 14 °C) remained high in the 8 kg range after day 3, which suggests there was little tenderisation occurring. Ferguson *et al.* (2000) reported 5.0 and 9.0 kg shear force for ES and non-stimulated beef LD muscle at 24 post-mortem using a similar WBS method. Whereas Devine *et al.* (1999) reported approximately 12 kg WBS for beef LD muscles entering rigor at 15 and 35 °C without ageing (1 h post rigor) and values of 4 and 8 kg after 7 days ageing respectively. The results obtained in this experiment for the 37 °C samples show a similar trend to that obtained by Devine *et al.* (1999) at 35 °C for days 1 and 7. However, the samples at 14 °C were expected to exhibit lower WBS values by day 3, especially when combined with electrically stimulation. According to previous reports, rapid tenderisation in beef should occur within 4 days post-slaughter

(Dransfield, 1994; Uytterhaegen *et al.*, 1994; Alarcon-Rojo and Dransfield, 1995; Taylor *et al.*, 1995; Koohmaraie, 1996).

There were no correlations between pH K and WBS at day 1 and 3 ($P>0.05$, Table 3.2), but there was a correlation between the pH K and the difference in WBS between days 1 and 3 ($P<0.01$, $r = -0.46$). The negative correlation indicates that faster pH decline corresponded to less tenderisation. This supports the suggestion that protein denaturation leads to less tenderisation during subsequent ageing (Warner, 1994). Furthermore, as discussed in the above section, PSE pork loses fluid rapidly early post rigor and then decreases in fluid loss after 24 h (Kim *et al.*, 1993; Joo *et al.*, 1995). Therefore, the negative correlation ($P<0.01$, $r = -0.45$) between the filter paper wetness on day 3 and WBS on day 3 (the lower the filter paper wetness at day 3, the higher the WBS) further confirms suggestions that rapid pH decline is associated with myosin denaturation and reduced tenderisation during ageing.

The MFI values were not different between the two temperatures on day 1 ($P>0.05$) but by day 3 the warm samples had a higher MFI than the hot samples ($P<0.05$). There was a correlation between the MFI on days 1 and 3 and the ATPase activity ($P<0.05$, $r = 0.36$, 0.49 respectively) which indicated that the lower the myofibrillar denaturation, the greater the fragmentation and hence tenderisation. There was also a negative correlation ($P<0.05$, $r = -0.37$) between MFI difference between day 1 and 3 and pH K. All of these results indicate that muscles with faster pH decline have higher protein denaturation and tenderise less during ageing than normal muscles.

The hypothesis is that PSE type muscle should produce more tender meat than normal muscle on day 1 post-slaughter but will exhibit a lack of tenderisation during ageing. The reason for this appears to be due to early activation of calpains pre-rigor but they also rapidly autolyse after activation (Simmons *et al.*, 1996) and hence little ageing occurs as shown by Locker and Daines (1975) and Devine *et al.* (1999). Locker and Daines (1975) showed that the WBS values for bovine *Rectus abdominis* muscles which entered rigor at 37 and 15 °C was 55.4 and 70.0N respectively at 24 h post-mortem. Devine *et al.* (1999) found that the WBS values for beef LD muscles entering rigor at 15 and 35 °C was not different (12.0 kg) at 1 h post rigor, but by day

7, the WBS value of the 15 °C samples was reduced to 4 kg compared to 8 kg for the 35 °C samples. In addition, using SDS-PAGE and Western blotting Warner *et al.* (1997) reported greater breakdown of nebulin and titin at 24 h post-mortem from PSE and reddish, soft and exudative (RSE) than reddish, firm and normal (RFN) and DFD (dark, firm, dry) pork loin samples. PSE and RSE samples also had lower WHC measurements (ie. higher drip loss, surface exudate and cook loss) and higher protein denaturation as measured by Ca^{2+}-ATPase activity and protein solubility compared to RFN and DFD samples. Furthermore, Hwang *et al.* (2001) showed that at 4 h post-mortem, the combination of low pH and high temperature at 1.5h post-mortem had the effect of lowering μ-calpain activity. At 24 h post-mortem, muscles which reached 32 °C at 1.5 h and had faster pH decline had lower μ-calpain activity than muscles that reached 24 °C at 1.5 h.

In this experiment, the hot samples would be expected to exhibit lower WBS values on day 1 due to accelerated tenderisation, but higher WBS values at day 3, relative to the warm samples. The 14 °C samples would be expected to have relatively lower WBS values than the 37 °C by day 3 since muscles entering rigor at 14 °C would have lower protein denaturation and minimum sarcomere shortening (Penny, 1977; Honikel *et al.*, 1981; Yates *et al.*, 1983; Honikel *et al.*, 1986; Judge *et al.*, 1989; Lawrie, 1991; Offer and Cousins, 1992). However, the high WBS values for both the 14 and 37 °C samples could not be due to muscle shortening since the sarcomere length only showed minimal shortening for both groups. Hence, the WBS results could not detect differences in tenderness due to the treatments at days 1 and 3.

Muscle Softness Evaluation by Instron Compression (IC) and Sensory Panel.

The IC at 5 mm depth for the anterior end on day 1 showed that the hot sample was softer than the warm sample ($P<0.05$, Table 3.3). In contrast, on day 3, the posterior end of the hot sample was firmer than the warm sample. Both of these results are consistent with the trends observed with the SE of the anterior and posterior ends on day 1 and 3. The IC loads of the anterior end on day 1 are 10 times greater than that of the posterior end on day 3 because two different compression methods were used. It is not possible to compare the IC results to literature since this is a new compression method.

There was no difference ($P>0.05$) in the SE of softness between the hot and warm samples for the anterior and posterior ends on days 1 and 3, but the anterior was softer than the posterior end on day 1 ($P<0.05$, statistical analysis not presented). The SE also found that the posterior end on day 3 was softer than the posterior end on day 1 ($P<0.05$), statistical analysis not presented). This shows that ageing results in softer muscles.

Correlation of Muscle Softness with pH K and Biochemical Properties

Table 3.2 shows that the IC of the anterior end on day 1 at 5 mm depth was correlated to the pH K ($P<0.05$, $r = -0.5$). The negative correlation indicated that the faster the pH decline (that is, higher pH K), the softer were the samples on day 1. Since the hot samples resulted in higher pH K than the warm, it could be concluded that high temperature caused faster pH decline and resulted in softer muscles. Furthermore, it is also possible that the long exposure to high temperature contributed directly to the softening of the muscles. The temperature decline rate has not been measured since the muscles were kept at two constant temperatures, 37 and 14 °C. The IC of the posterior end on day 3 was correlated ($P<0.05$, $r = 0.41$) to the pH K at 8 mm depths (result not shown) which suggests that the faster the pH decline (higher pH K), the firmer were the muscles on day 3. This trend is consistent with the hot samples on day 3 being firmer than the warm samples. The SE of softness (anterior day 1 and posterior days 1 and 3) did not correlate with the pH K ($P>0.05$) and perhaps this is not surprising since the SE did not detect differences in softness between the hot and warm muscles.

Table 3.3 shows significant correlations between the IC of the anterior end on day 1 with the L* and a* values, the difference in filter paper wetness between day 1 and 3, the ATPase activity and the sarcoplasmic protein solubility ($r = -0.53$, -0.42, -0.58, 0.54, 0.38 respectively, $P<0.05$ for all). The negative correlation between the IC and the difference in filter paper wetness indicates that the softer the muscle was on day 1, the greater was the surface exudate on day 1. The SE of softness also showed correlations with drip loss ($r = -0.34$, $P<0.05$) and filter paper wetness on days 1 and 3 ($r = -0.44$ and -0.61 respectively, $P<0.01$) which confirms the IC results that higher

surface exudate and drip loss corresponded to softer muscles. There was no correlation ($P>0.05$) between the IC data and the WBS or MFI measurements. Only the WBS value on day 3 was correlated with the SE of softness which shows that higher WBS value on day 3 (less tenderisation) resulted in firmer muscles.

As found earlier in this chapter, rapid pH decline and high temperature increase protein denaturation and water loss. The above correlations of protein denaturation and WHC measurements with muscle softness show that rapid pH decline and high temperature also cause softer muscles. Previous studies (Penny, 1969; Locker and Daines, 1975; Fischer and Hamm, 1980; Honikel *et al.*, 1981; Offer, 1991; van Lack and Solomon, 1994) that have reported the relationships between rapid pH decline and high temperature with increased protein denaturation and water loss "observed" softer muscles but did not actually quantify and correlate the muscle softness with these parameters. The correlation between the WBS value on day 3 with the SE of softness (that is, the higher the WBS on day 3, the firmer the meat) tends to support the hypothesis that lack of tenderisation during ageing results in firmer muscles. However, the lack of correlation between muscle softness and the WBS or MFI data on day 1 does not support the hypothesis that accelerated tenderisation may contribute to increased muscle softness. This could due to the lack of differences in WBS and MFI values between the 37 and 14 °C samples on day 1.

Correlation of Muscle Softness with Fat Content and Sarcomere Length

Table 3.3 shows that there was no correlation between fat content and the IC at 5 mm depth of the anterior end on day 1 ($P>0.05$). However, at a higher compression depth of 11 mm, there was a negative correlation ($P<0.05$, $r = -0.43$, result not shown) which suggests that the higher the fat content of the muscle, the softer is the muscle. This correlation was not as high as the correlation between the pH K and muscle softness and hence suggests that marbling did not affect muscle softness as much as the pH K in this experiment. This could be due to the fact that the experiment did not contain muscles having a large range of fat content, or that the pH K difference between the 37 and 14 °C was much greater than the fat content range in the muscles.

The SE of softness for the posterior end on day 1 was positively correlated to the fat content ($P<0.05$, $r = 0.45$). Wood (1991) suggested that since intramuscular fat is less dense than the myofibrils, it would be expected that the higher the fat content, the softer the muscle would be. Therefore, the correlation between the IC data at 11 mm depth and the fat content would be the more reliable relationship. In addition, the IC had detected differences in softness between the hot and the warm samples, whereas the SE of softness did not. However, since the IC method is still at a developmental stage, it's results are accepted with caution.

3.4 Conclusion

It was shown that the pH decline rate in the LD muscle was highly affected by early post-mortem chilling temperature with the high temperature having nearly double the pH decline rate (as measured by pH K) as that of the moderate temperature treatment. There was no correlation between the pH decline rate and the sarcomere length nor was there a difference in the sarcomere length between the high and medium temperature treatment even though the muscle at high temperature had been expected to show heat shortening. The IC data was positively correlated with the fat content of the muscles indicating that the higher the fat content, the softer the muscle.

The results showed that the high temperature treatment caused faster pH decline and subsequently greater protein denaturation and lower WHC than the moderate temperature. This confirms that protein denaturation and WHC are directly related and are influenced by rapid pH decline and high temperature. The IC data showed that the high temperature treatment caused softer muscle than the moderate temperature. A negative correlation between the IC and the pH decline rate supported the hypothesis that rapid pH decline and high temperature early post-mortem lead to softer muscles.

There were few differences in tenderness as measured by WBS and MFI between the high and moderate temperature treatments. Only the MFI data showed that muscles from the moderate temperature treatment aged more than the high temperature treatment. Hence the expected acceleration in tenderisation by day 1 for muscles with

high temperature treatment was not observed. The negative correlation between the pH decline rate and the difference in MFI and the difference in WBS values between days 1 and 3 tend to indicate that faster pH decline leads to less tenderisation. Hence it could be concluded that in conditions where faster pH decline causes greater protein denaturation and lower WHC, tenderisation during ageing is reduced. In addition, the hypothesis that high temperature and rapid pH decline early post-mortem should result in accelerated break down of myofibrillar proteins was not supported.

There was little correlation between the tenderness early post-mortem as measured by WBS and MFI on day 1 and muscle softness. Since there was also no difference in tenderness between the high and moderate temperature treatments, the hypothesis that accelerated tenderisation leads to softer muscles early post-mortem was not supported. Instead it was observed that the moderate temperature treatment resulted in greater tenderisation and softer muscles during ageing than the high temperature treatment. Therefore, it could be concluded that early development of soft muscles is due to higher protein denaturation and poor WHC as a result of high temperature and rapid pH decline and not due to accelerated proteolysis.

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Table 3.1: The effects of early post-mortem temperature (hot 37 °C vs. warm 14 °C) on meat quality, protein denaturation, proteolysis, WHC and on the softness measurements on beef *Longissimus dorsi* muscles after 1 or 3 days ageing. The means are least squares mean.

Physical measurements	Hot	Warm	SED	P value
pH K (pH unit/h)	0.37	0.22	0.04	0.01
Ultimate pH	5.47	5.46	0.23	0.96
CIE – L*	37.1	33.8	0.88	0.01
CIE – a*	18.6	16.9	0.66	0.01
CIE – b*	8.60	5.60	0.81	0.01
Fat content (% wet weight)	1.59	1.63	0.26	0.89
Sarcomere length (μm)	1.70	1.73	0.06	0.59
Drip loss (%)	1.35	1.08	0.10	0.01
Filter paper wetness - day 1 (mg/cm^2)	4.61	2.16	0.23	0.01
Filter paper wetness - day 3 (mg/cm^2)	4.11	3.27	0.31	0.01
Difference in filter paper wetness (day 3 – day 1)	-0.50	1.10	0.23	0.01
Cook loss - day 1 (%)	23.4	21.0	1.01	0.02
Cook loss - day 3 (%)	29.4	26.7	1.12	0.03
Difference in cook loss (day 3 – day 1)	5.22	5.71	1.35	0.72
Purge - day 3 (%)	1.18	1.06	0.14	0.39
Protein denaturation & Proteolysis				
Myofibrillar ATPase (μmol Pi/mg protein/min)	0.24	0.32	0.01	0.01
Sarcoplasmic protein solubility (mg/g)	71.7	79.2	5.4	0.18
Warner-Bratzler shear (WBS) - day 1 (Kg)	7.65	8.76	0.87	0.21
Warner-Bratzler shear - day 3 (Kg)	8.13	8.32	0.85	0.82
Difference in WBS (day 3 – day 1)	0.48	-0.44	0.98	0.36
Myofibrillar fragmentation index (MFI) - day 1	41.3	40.3	3.10	0.75
Myofibrillar fragmentation index - day 3	45.6	52.1	2.55	0.02
Difference in MFI (day 3 – day 1)	4.20	11.7	3.35	0.03
Muscle softness				
Sensory evaluation*: Anterior Day 1	3.71^a	4.36^a	0.38	0.10
Posterior Day 1	5.10^b	5.22^b	0.45	0.79
Posterior Day 3	4.41^a	3.88^a	0.35	0.14
Instron compression$^+$: Anterior Day 1 (g)	270	350	30	0.02
Posterior Day 3 (g)	19.4	12.5	1.9	0.01

*scoring scale, 1 = extremely soft, 8 = extremely firm

$^+$ Instron compression load (g) at 5.0 cm compression depth

^{ab} values lacking common superscript within row and across column are significantly different at $P<0.05$

SED = standard error of difference

Table 3.2: Pearson correlation coefficients (r) between various biochemical and physical measurements of beef *Longissimus dorsi* muscles to show their relationships due to the effects of post-mortem high (hot 37 °C) and medium (warm 14 °C) temperature control.

	pH K	CIE – L*	CIE – a*	ATPase	SPS	Purge day 3	Drip loss	FPW day 1	FPW day 3	FPW differ	SL	Fat content	CL day 1	CL day 3	CL differ	WBS day 1	WBS day 3	WBS differ	MFI day 1	MFI day 3
CIE - L	0.20																			
CIE - A	0.36*	0.52**																		
ATPase	-0.49**	-0.49**	-0.63**																	
SPS	-0.07	-0.25	-0.46**	0.52**																
Purge day 3	0.17	0.05	0.42*	-0.29	-0.07															
Drip loss	0.24	0.43*	0.44**	-0.48**	-0.13	0.39*														
FPW day 1	0.38*	0.68**	0.47**	-0.64**	-0.22	0.22	0.54**													
FPW day 3	-0.03	0.50**	0.24	-0.37*	-0.05	0.25	0.46**	0.67**												
FPW differ	0.53**	0.44**	0.41*	-0.51**	-0.26	0.06	0.29	0.72**	-0.04											
SL	-0.11	0.33	0.55**	-0.29	-0.28	0.22	0.22	0.03	0.25	-0.20										
Fat content	0.29	0.33	0.06	0.01	-0.15	-0.24	-0.14	-0.11	-0.35*	0.18	0.10									
CL day 1	0.41*	0.27	0.56**	-0.65**	-0.41*	-0.06	0.21	0.29	-0.03	0.42*	0.10	0.11								
CL day 3	0.25	0.12	0.06	-0.25	0.13	-0.12	0.04	0.26	0.08	0.28	-0.06	-0.01	0.31							
CL differ	-0.14	-0.11	-0.35*	0.31	0.42*	-0.05	-0.11	-0.03	0.14	-0.17	-0.01	-0.06	-0.52**	0.65**						
WBS day 1	-0.14	-0.38*	-0.37*	0.16	0.12	-0.26	-0.24	-0.33	-0.33	-0.13	-0.32	-0.28	-0.06	0.25	0.23					
WBS day 3	-0.32	-0.33	-0.01	-0.10	-0.05	0.08	-0.03	-0.22	-0.45**	0.13	-0.38*	-0.11	0.41*	0.20	-0.18	0.34*				
WBS differ	-0.46**	-0.06	-0.32	0.23	0.15	-0.30	-0.19	-0.11	0.09	-0.22	0.03	-0.15	-0.40*	0.05	0.35*	0.60**	-0.55**			
MFI day 1	0.23	-0.02	-0.25	0.36*	0.28	-0.05	0.09	0.07	-0.04	0.13	-0.33	0.03	-0.31	-0.03	0.16	0.35*	-0.02	-0.30		
MFI day 3	-0.22	-0.38*	-0.29	0.49**	0.31	-0.04	-0.13	-0.29	-0.22	-0.18	-0.14	0.02	-0.22	-0.25	-0.03	-0.14	-0.24	0.08	0.26	
MFI differ	-0.37*	-0.28	-0.01	0.07	-0.01	0.01	-0.18	-0.28	-0.14	0.25	0.17	-0.01	0.10	-0.18	-0.16	0.20	-0.17	0.32	-0.66**	0.55**

* significant at $P<0.05$, ** significant at $P<0.01$, number of samples n = 34
ATPase = myofibrillar Ca^{2+}-ATPase, SPS = sarcoplasmic protein solubility
FPW = filter paper wetness, FPW differ = difference in filter paper wetness (day 3 – day 1)
SL = sarcomere length, CL = cook loss, CL differ = difference in cook loss (day 3 – day 1)
WBS = Warner-Bratzler shear, WBS differ = difference in WBS (day 3 – day 1)
MFI = myofibrillar fragmentation index, MFI differ = difference in MFI (day 3 – day 1)
Day 1 = 1 day post-slaughter, Day 3 = 3 days post-slaughter,

Table 3.3: Pearson correlation coefficients (r) between muscle softness as measured by sensory evaluation (SE) and Instron compression (IC) at 5 mm compression depth with various biochemical and physical measurements of beef *Longissimus dorsi* muscles.

	pH K	CIE – L*	CIE – A*	ATPase	SPS	Purge day 3	Drip loss	FPW day 1	FPW day 3	FPW differ	SL	Fat content	CL day 1	CL day 3	CL differ	WBS day 1	WBS day 3	WBS differ	MFI day 1	MFI day 3	MFI differ
IC:Anterior day 1	-0.50**	-0.53**	-0.42*	0.54**	0.38*	-0.12	-0.10	-0.31	0.19	-0.58**	-0.17	-0.32	0.33	-0.19	0.19	0.22	-0.11	0.26	0.01	0.27	0.19
IC:Posterior day 3	0.34	0.42*	0.17	-0.22	-0.09	0.02	-0.06	0.36*	-0.04	0.51**	-0.41*	0.20	0.19	0.41*	0.06	0.16	0.19	-0.03	0.08	-0.35	-0.33
SE:Anterior day 1	-0.24	-0.16	-0.12	0.02	-0.25	-0.11	-0.34*	-0.44**	-0.30	-0.31	-0.081	0.25	0.11	-0.23	-0.31	0.27	0.38*	-0.08	-0.39*	-0.21	0.17
SE:Posterior day 1	0.04	-0.06	-0.14	0.02	-0.24	-0.18	-0.24	-0.32	-0.61**	0.13	-0.39*	0.45*	0.27	0.04	-0.25	0.24	0.58**	-0.27	-0.12	-0.17	-0.03
SE:Posterior day 3	0.09	0.32	0.10	-0.30	-0.38*	0.10	0.12	0.11	-0.13	0.26	-0.20	0.34	0.36	0.13	-0.20	0.04	0.47**	-0.35	-0.20	-0.46*	-0.18
Diff in posterior	0.05	0.50**	0.33	-0.40*	-0.10	0.40*	0.52**	0.62**	0.77**	0.12	0.35	-0.28	0.02	0.10	0.14	-0.32	-0.31	-0.01	-0.06	-0.30	-0.18

* significant at $P<0.05$, ** significant at $P<0.01$, number of samples n = 30

pH K = pH decline rate constant

ATPase = myofibrillar Ca^{2+}-ATPase

SPS = sarcoplasmic protein solubility

FPW = filter paper wetness, FPW differ = difference in filter paper wetness (day 3 – day 1)

SL = sarcomere length

CL = cook loss, CL differ = difference in cook loss (day 3 – day 1)

WBS = Warner-Bratzler shear, WBS differ = difference in WBS (day 3 – day 1)

MFI = myofibrillar fragmentation index, MFI differ = difference in MFI (day 3 – day 1)

Diff in posterior = difference in softness (day 1 – day 3) in the posterior end

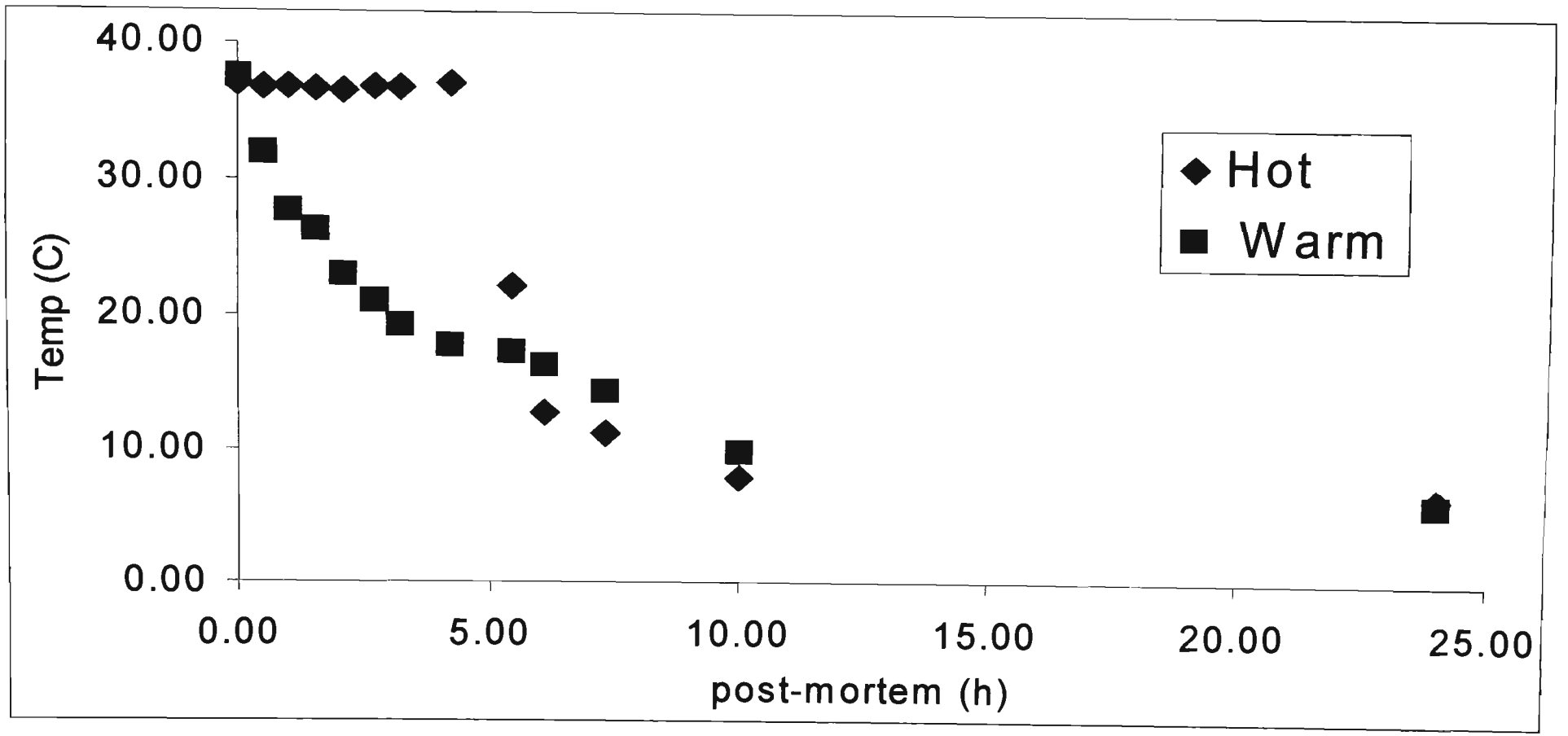

Figure 3.1: Temperature decline curves of beef *Longissimus dorsi* muscles incubated in water bath at hot and warm temperatures (37 vs 14 °C) early post-mortem until rigor (pH< 5.8) and then transferred to a 2 °C ice-bath.

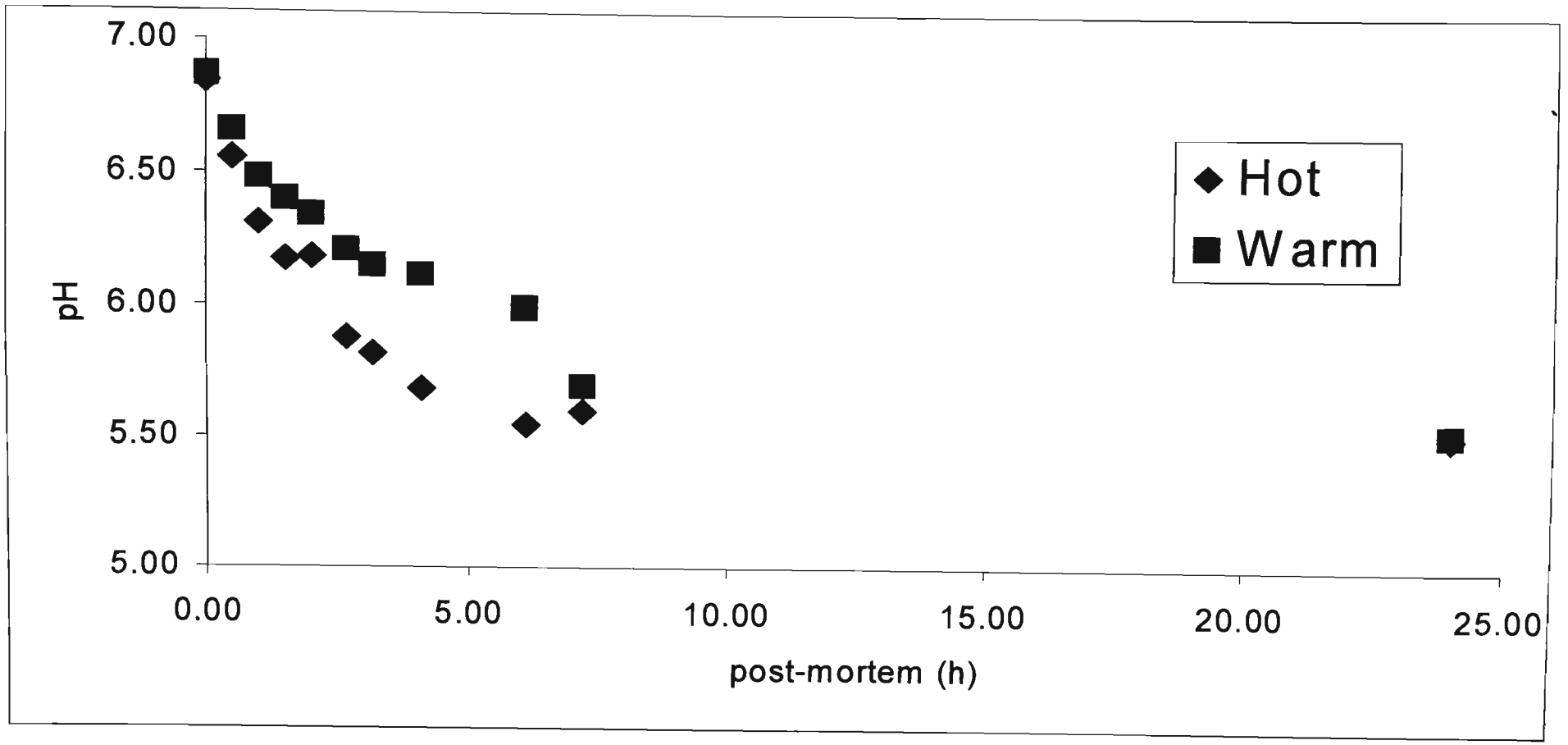

Figure 3.2: The effects of early post-mortem hot and warm temperatures (37 vs 14 °C) on the pH decline of beef *Longissimus dorsi* muscles.

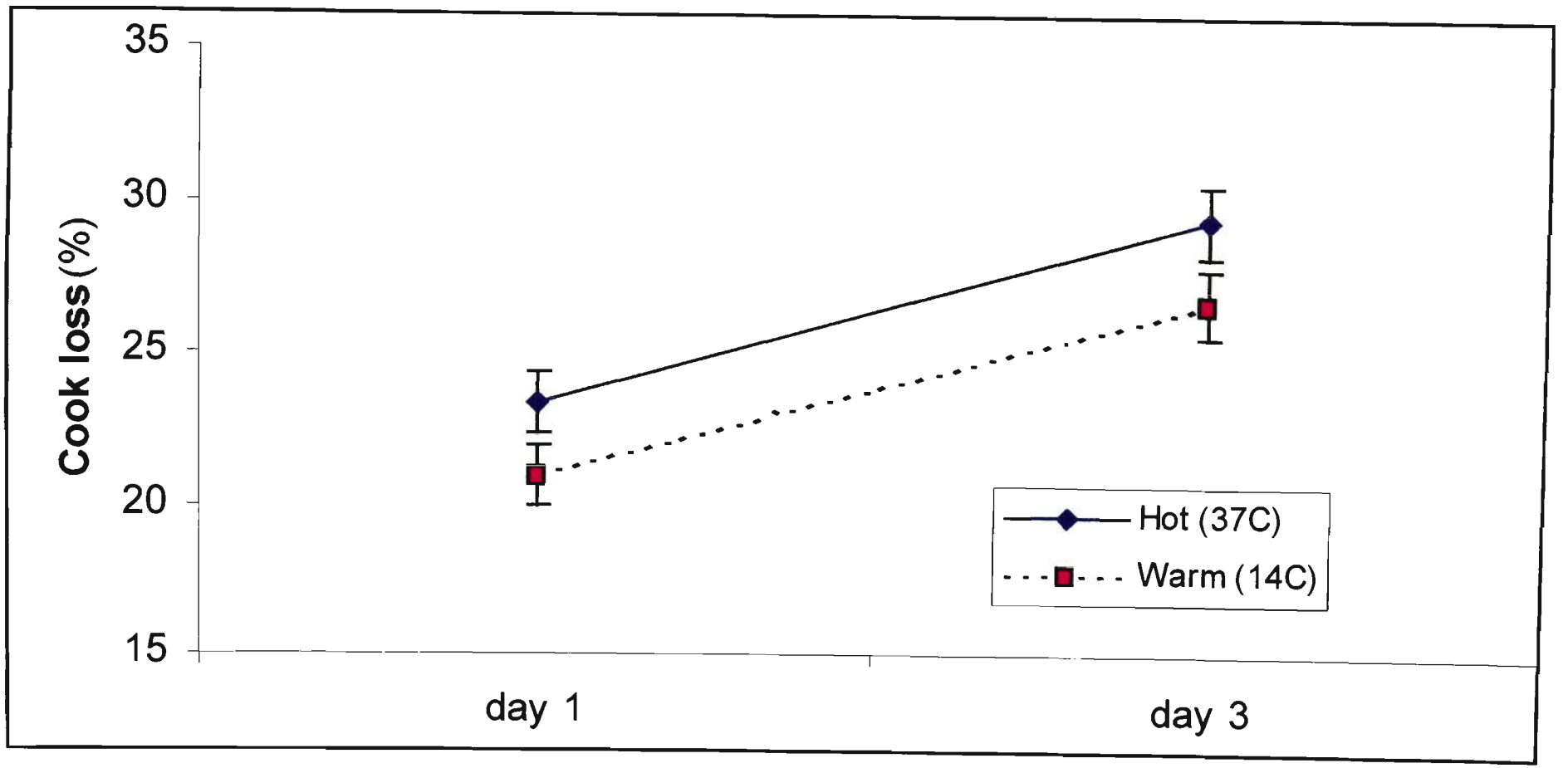

Figure 3.3: The effects of early post-mortem hot and warm temperatures (37 vs 14 °C) on the amount of cook loss at 1 and 3 days post-slaughter of beef *Longissimus dorsi* muscles.

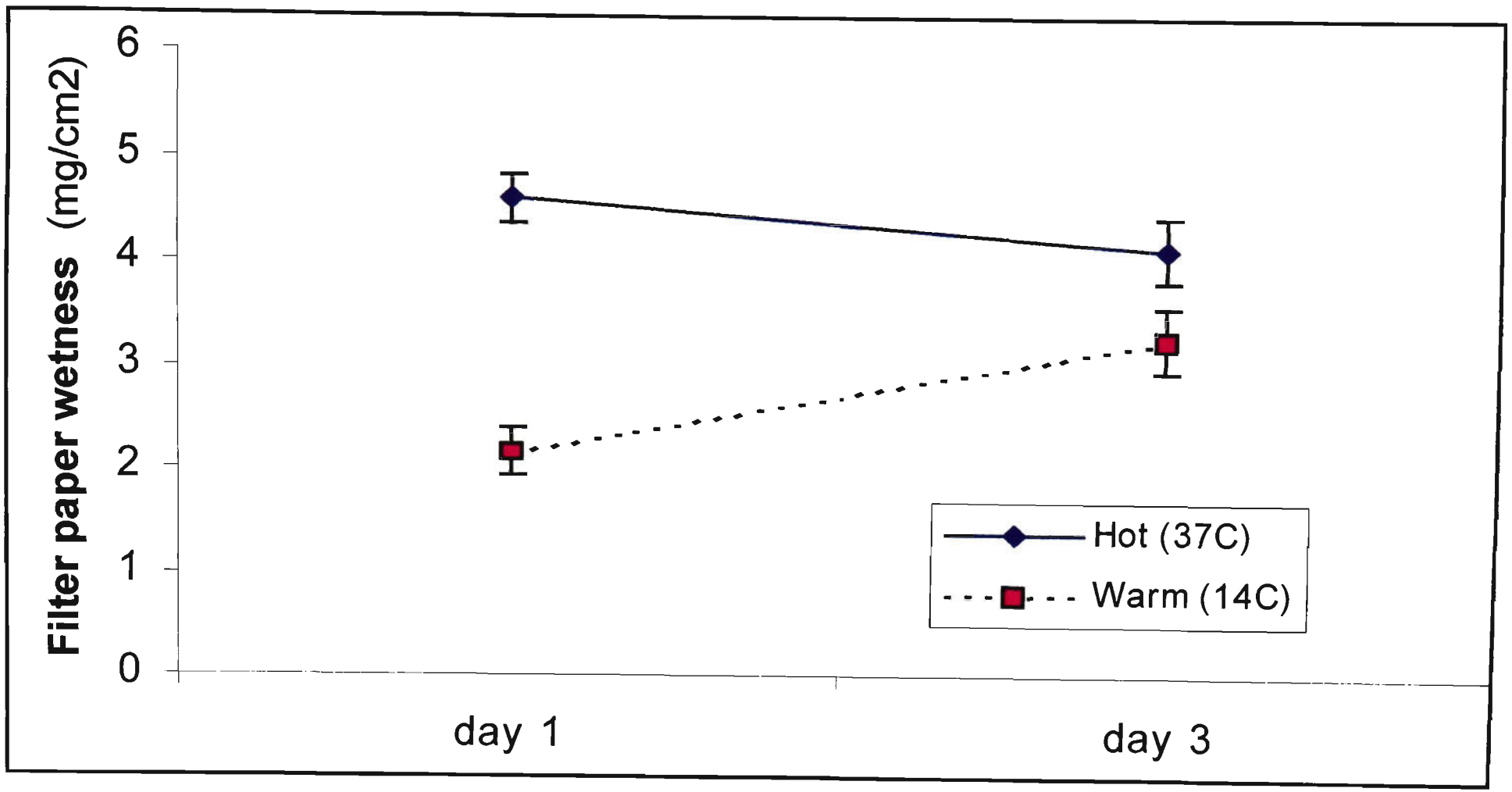

Figure 3.4: The effects of early post-mortem hot and warm temperatures (37 vs 14 °C) on the amount of drip at 1 and 3 days post-slaughter of beef *Longissimus dorsi* muscles.

CHAPTER 4

THE INFLUENCE OF TEMPERATURE AND pH DECLINE RATES AND FIBRE TYPE ON THE SOFTNESS AND WATER HOLDING CAPACITY OF TWO BEEF MUSCLES

4.1 Introduction

Pale, Soft and Exudative meat is a well-known quality defect in the pork industry (Judge *et al.*, 1989; Lawrie, 1991). However, under certain cases beef also exhibits the conditions in a milder form with lighter colour, softer texture and increased surface exudate than normal (Locker and Daines, 1975; Fischer and Hamm, 1980). The causes of this problem in beef are strongly suggested to be due to both rapid pH decline and the attainment of low pH while the muscle temperature is high (Tarrant and Mothersill, 1977; Offer, 1991; Penny, 1969), that is, similar to the causes in pork.

Fischer and Hamm (1980) found in beef carcasses that 64% of the PM muscles had pH less than 6 by 30 mins post-slaughter. The characteristics of the affected PM muscles were similar to PSE pork but less pronounced. Similarly, beef muscles at a greater depth in the carcass (e.g. deep in the butt) which are at a higher temperature for longer period are more likely to exhibit the PSE condition (Tarrant and Mothersill, 1977; Offer, 1991). Tarrant and Mothersill (1977) found that different beef muscles of the round such as the AD, SM, ST and BF had different pH decline rates. The ST had the fastest pH decline, followed by the AD, BF and SM muscles. The reasons the muscles differ in pH decline rate could be due to their different composition of fibre types and different depths from the carcass surface causing differences in the temperature decline. These results also showed that the highest pH decline rate did not result in the highest amount of drip. The ST muscle reached pH 6 in 2.8 h while the SM reached pH 6 in 4.1 h and yet the ST muscle produced 16.7% drip compared to 19.4% drip in the SM. One of the reasons for this difference in drip could be associated with structural characteristics of the muscles rather than linked to the pH and temperature decline rates. . However, the authors did not provide data on protein denaturation or sarcomere length differences between muscles to explain the differences in drip loss. In addition, it was unclear from their results if the muscle temperature had an impact on muscle softness and WHC.

An important difference in muscle fibre characteristics that could affect the WHC and softness, is that muscles containing predominantly red fibre type have lower amount of calpains, hence they appear to tenderise more slowly than white muscles (Honikel *et al.*, 1981; Boakye and Mittal, 1993; Cheah *et al.*, 1995). Some authors have suggested that accelerated rigor onset associated with rapid pH fall may cause early post-mortem proteolytic breakdown of muscle proteins that can influence both the WHC and the softness (Boakye and Mittal, 1993; Cheah *et al.*, 1995). If that is the case, then a muscle containing predominantly white fibres should exhibit greater tenderisation and perhaps early development of softer texture and poorer WHC.

The incidence of soft-watery meat is usually associated with low WHC, but there is little data to show that poor WHC is the cause of the excessive softness in muscles. It is possible that soft muscles are not directly related to low WHC but rather an indication of other structural changes that cause the excessive softness. Hence it is possible that soft textured meat can have high WHC.

The hypotheses for this experiment are;

1) high muscle temperature leads to softer muscles,
2) a muscle containing predominantly white fibres should exhibit greater tenderisation and early development of softer texture and poorer WHC.
3) soft textured muscles can have high WHC.

The aims of this experiment were to investigate 1) how the temperature and pH decline rates and muscle fibre types affect muscle softness, WHC and post-mortem biochemical changes (protein denaturation, sarcomere shortening and proteolysis) of beef muscles and 2) whether muscle softness is dependent on the WHC.

4.2 Materials and Methods

Animals

Twenty grass-fed Angus x Hereford steers approximately 22 months old were transported 500 km to the Food Science Australia Laboratory in Brisbane and kept together on pasture for one week before slaughter. Groups of four steers were killed over five days. Live weights and carcass weights were 353 ± 21 and 182 ± 9 kg, respectively, with an average 12/13th fat thickness of 2.9 ± 0.5 mm.

Experimental Design

Alternate carcasses were ES 3 mins post-mortem via a nostril/rectal probe with low voltage (45 volts, 100 ms on and 12 ms off, 36 Hz with 500 mA current) for either 10 or 40 secs. The carcasses were split in half and the right side chilled rapidly in a 1 °C chiller equipped with a blast fan (1.25 m/s air speed, 86% humidity), and sprayed with an ice slurry every 5 to 10 mins for the first hour in the chiller. The left side was chilled slowly at about 18 °C for 2 h post-mortem before being placed in a slow chiller (6 °C, 0.45 m/s air speed, 83% humidity) for a minimum of 7 h. Thereafter all sides were moved to the fast chiller for overnight storage. Thus the treatments were two different chill rates, rapid and slow, and two ES times, 10 and 40 secs.

Temperature and pH Measurements

Muscle temperature was logged (Cox Tracer, USA) using thermocouples inserted in the centre of the LD between the 3rd and 4th lumbar vertebrae and in the anterior of the SM muscles at 3-4 cm depth from approximately 50 mins postmortem, at 5 mins interval until the following day. Muscle samples (500 mg) were collected every 15 mins for 2 h post-mortem and then every hour for the next 5 h from the caudal portion of the LD and SM muscles and immediately placed in liquid nitrogen. The pH of these frozen samples was determined later by homogenising in an iodoacetate-KCl solution (pH, 6.97) at a temperature of 18 to 20 °C (Bendall, 1973). The 24 h pH was measured with a portable pH meter (Hanner Instrument HI9024, USA) equipped with a glass probe electrode and temperature compensation (Prometary Ltd. Ionode electrode IJ42, Australia).

Sampling

The LD and SM muscles were boned out the next day, trimmed of surface fat and connective tissues and subjectively evaluated for softness using an untrained sensory panel as described in Chapter 2.2.2. The muscles were then cut transversely across the fibre for the SM and across the length of the LD for surface exudate analysis using filter paper and colour measurement (methods described below). Thin cross sectional slices were then removed from the center and ends of each muscle to ensure representative sampling for biochemical assays (myofibrillar ATPase, protein solubility and MFI). Samples were removed for the WBS (222 ± 21 g), drip loss (81 ± 7 g), MFI (80 ± 5 g) and sarcomere length (50 ± 5 g) tests. Muscle sections for MFI, cook loss and WBS were vacuum packaged, randomly allocated to ageing of 1,3 or 14 days for the SM and 1,3, 7 or 14 days for the LD muscles stored at 4 °C and then frozen at –20 °C. All the procedures used for the biochemical and physical tests are the same as that described in Chapter 2.2.1 and 2.2.3, unless otherwise specified.

Meat blocks of approximately 5x6x8 cm from each muscle were allocated for further softness evaluation by the sensory panel and IC as described in Chapter 2.3 using 10 mm compression depth. Blocks of SM muscles weighing approximately 300 - 400 g were vacuum packaged, chilled at 4 °C for 14 days and the softness evaluated. These samples were also analysed for purge.

Sarcoplasmic Protein Solubility – The method is described in Chapter 3

Total Protein Solubility

The total protein solubility assay was conducted with minor modification according to the method of Helander (1957). Approximately 20 g of fresh muscle sample, free of fat and connective tissue was finely chopped. Duplicate 1.0 g of fresh finely chopped sample was added to 25 mL of cold freshly mixed 1.1 M KI/0.1 M sodium phosphate buffer at pH 7.5, homogenised with an Ultra-Turrax T25 (Janke & Kunkel, Germany) for two 8.0 secs bursts at maximum speed while keeping the samples cool in ice-water. The solution was filtered through a 12.5 cm diameter N°1 Whatman filter paper for 1.0 hour. The protein concentration of the filtrate was determined in duplicate by mixing 4.5 mL of biuret reagent (36 mM $CuSO_4$, 3% (v/w) NaOH, 127 mM sodium tartate) to 0.5 mL of the filtrate and reading the absorbance at 540 nm after half an hour (Gornall *et al.*, 1949). BSA (Sigma,

Fraction V, A-6793, St. Louis) in the range of 0 – 5 mg/mL was used as the standard. A correction blank for back ground absorbance was included by mixing 4.0 mL of a solution containing 127 mM sodium tartate and 3% (v/w) NaOH to 1.0 mL of the filtrate and measuring the absorbance at 540 nm shortly after mixing. This was to correct for absorbances from highly colour proteins such as haemoglobin that could increase the background absorbance. The total protein solubility was expressed as mg/g meat.

Statistical Analyses

The pH and temperature decline were modeled using an exponential function; $pH(t) = pH\infty + (pH_0 - pH\infty)e^{-Kt}$, where pH(t) = pH at time t, pH_0 = pH at time 0, $pH\infty$ = pH at time ∞ and K was the rate constant of pH decay. The rate constant K was used as a measure of pH and temperature decline rates. Log transformations were performed on the temperature and pH decline rates constant K since it showed increasing variance with increasing values. This was conducted in order to provide an unbiased estimate of variance. The WBS values at 1, 3, 7 and 14 days of ageing for the LD and 1, 3 and 14 days of ageing for the SM muscles were also modeled using the exponential equation to determine the ageing rate constants K. In addition, the difference between day 1 and day 14 in the WBS and MFI values were calculated to measure the effects of pH and temperature decline on ageing. To test for significant differences, all the parameters were analysed by ANOVA using General Linear Model of SPSS statistical software (version 9) (SPSS Inc. Chicago, Illinois, USA).

4.3 Results and Discussion

Temperature and pH decline rates

Table 4.1 and Figures 4.1, 4.2, 4.3 and 4.4 show the effects of ES and chilling rates on the pH and temperature decline of the LD and SM muscles. As expected, muscles with 40 secs ES had faster pH decline rate than 10 secs ES as measured by the pH rate constant K ($P<0.01$). The 40 sec ES had a faster pH decline than 10 sec ES in the LD but not in the SM muscle (muscle x ES interaction, $P<0.001$). The pH K was lower for rapid chilling than for slow chilling for the 10 secs stimulation, but the pH K for the 40 sec ES was similar and high for slow and rapid chilling (ES x chill rate interaction, $P=0.06$).

Table 4.1 also shows that the temperature decline was much faster with rapid chilling compared to slow chilling in LD but not in the SM muscle (muscle x chill rate interaction,

P<0.001). As anticipated the ES x chill rate interaction for the Temp K was not significant (P>0.10) and the ES treatment also had no direct effect on Temp K (P>0.10). These results show that the ES and chilling treatment had more effect on the pH and temperature decline rates in the LD than on the SM muscles.

According to Hunt and Hedrick (1977) and Devine *et al.* (1984), the LD muscle contains more slow-twitch-oxidative (type I) fibres than the SM and responds less intensively to ES than fast-twitch-oxidative-glycolytic (type IIA) or fast-twitch-glycolytic (type IIB) fibres. Den Hertog-Meischke *et al.* (1997) also found that ES caused faster pH decline in the SM than the LD beef muscles. In this experiment, the SM had a relatively similar pH decline for both ES times whereas the LD responded with a much faster pH decline to the 40 secs ES compared to the 10 secs stimulation. This suggests that the LD was more affected by ES than the SM muscle. It is possible that the SM did not receive the same amount of ES as the LD muscles because the ES was done on the carcass where the electrical pulse may not have reached the SM as much as it did the LD muscles.

Physical Measurements

Table 4.1 also shows that the ultimate pH of rapidly chilled muscle was slightly higher than slowly chilled muscle (P<0.05), but there was no difference in the ultimate pH due to ES (P>0.05). There was also no difference in the ultimate pH between the LD and SM muscles (P>0.05) and this agrees with den Hertog-Meischke *et al.* (1997) and Babiker and Lawrie (1983) who also reported no difference in the ultimate pH between the stimulated SM and LD or between the stimulated and non stimulated muscles.

Surface lightness value (CIE - L) was higher for slow chilling than rapid chilling (P<0.01), but there was no difference between the two ES times (P>0.05). Generally, the SM muscle was redder (higher a* value) than the LD muscle (P=0.057) and slowly chilled muscles were redder than rapidly chilled muscles (P < 0.01).

The sarcomere length was not different due to the stimulation, the chill rate or muscle (P>0.05). LD beef muscle normally has a sarcomere length of 1.89 - 2.0 μm (Cross *et al.*, 1981; Honikel *et al.*, 1986; Devine *et al.*, 1999) and hence the sarcomere lengths measured in this experiment are not shortened since they were in this range. Therefore, any observed differences in the WHC, softness or tenderness could not be due to the sarcomere lengths.

Water Holding Capacity Measurements

Table 4.2 shows that slow chilling resulted in more drip loss than rapid chill ($P<0.05$) while ES time had no effect ($P>0.05$) on the SM and LD muscles. The SM with slow chill and 40 secs ES tended to give the highest drip loss while the LD with rapid chilling and 10 secs ES tended to give the lowest (muscle x chill rate x ES interaction, $P=0.086$). This suggests that drip loss was most affected by a combination of rapid pH decline and high temperature. Overall, the SM had more drip loss than the LD muscle ($P<0.001$) and this result agrees with the findings of den Hertog-Meischke *et al.* (1997) who reported that the SM muscle had 3.2% drip compared to the LD muscle with 1.7% drip. A previous study by Bruce and Ball (1990) also showed that drip loss in bovine ST muscle had a big difference in drip loss due to chilling (0.84 – 2.33%, rapid vs slow chilling respectively) and a much smaller effect of ES (1.16 – 2.01%, no ES vs with ES respectively).

Overall, the filter paper wetness values showed similar treatment effects to the drip loss values. The filter paper wetness values was higher for slow chilling than rapid chilling ($P<0.001$) and for 10 secs ES the SM had higher filter paper wetness values than the LD muscle (muscle x ES interaction, $P<0.05$). Overall, the filter paper wetness value for the SM was much greater than the LD muscle ($P<0.001$), and these results are similar to those reported by den Hertog-Meischke *et al.* (1997) of 22.4 mg of water for the LD and 42.3 mg for the SM muscle.

The day 14 purge for the SM muscle was not affected by either stimulation, chill rate or muscles. This lack of difference was probably because there was only small differences in the pH and temperature decline rates in the SM muscles as shown in Table 4.1. Purge was not measured for the LD muscle.

Tarrant and Mothersill (1977) found that although the ST muscles reached pH 6 in 2.8 h and the SM in 4.1 h, the SM had higher drip loss (19.4%) than the ST muscle (16.7%). Similar trend was found in this experiment. Although the SM had slower pH decline rates than the LD muscle, it had higher drip loss and filter paper wetness. The chilling treatments resulted in greater differences in drip loss and filter wetness than the ES treatments and hence suggest that the temperature decline rates had a greater influence on the WHC than the pH decline rates.

Protein Denaturation Measurements

Table 4.2 also shows that, as expected, slow chilling resulted in lower myofibrillar ATPase activity (indicates greater myosin denaturation) than rapid chill ($P<0.05$) while 40 secs ES resulted in lower ATPase activity than 10 secs ($P<0.001$). The SM had lower myosin ATPase activity than the LD muscle ($P=0.058$). Both the ES and chilling treatments rates caused differences in myofibrillar ATPase results, but the range in ATPase activity was larger with ES (0.53 to 0.60 μmol Pi/mg/min) than chill rates (0.55 to 0.59 μmol Pi/mg/min). The LD and SM muscles with slow chilling and 40 secs ES tended to result in the lowest myofibrillar ATPase activity (muscle x chill rate x ES interaction, $P=0.088$) compared to the values for other treatment combination. These results agree with previous studies which have found lower Ca^{2+}-ATPase activity under rapid pH decline and high temperature in both beef and pork muscles (Tarrant and Mothersill, 1977; Fischer and Hamm, 1980; den Hertog-Meischke *et al.*, 1997; Warner *et al.*, 1997).

Surprisingly, the sarcoplasmic and total protein solubility was not different ($P>0.05$) due to ES or chilling treatments. As the chilling and ES treatments influenced the myofibrillar ATPase activity, the sarcoplasmic and total protein solubility were also expected to show differences due to treatments. However, with the sarcoplasmic protein solubility there was a consistent increase in protein concentration from the least denaturing condition (10 secs ES and rapid chill) to the most denaturing condition (40 secs ES and slow chill). The SM had lower ($P<0.05$) total protein solubility than the LD muscle but this was not observed with the sarcoplasmic protein solubility. This was consistent with the SM having lower myofibrillar ATPase activity than the LD muscle. The reason for this lack of difference in protein solubility between treatments is probably due to the lack of precision in the method.

In summary, the SM had greater drip loss and filter paper wetness and lower myofibrillar ATPase activity and total protein solubility than the LD muscle. These 4 assays all indicated that the SM muscle underwent more denaturation. This corresponds to the lower WHC in the SM compared to the LD muscle. However, the range of myofibrillar ATPase activity was larger with the LD than the SM muscle due to the limited impact of treatments in the SM. This suggests that although the SM on average is more susceptible to denaturation than the LD, the fastest pH decline and slowest temperature decline caused the highest myofibrillar denaturation in the LD. Therefore, these results confirm previous

studies that the most severe denaturing conditions are rapid pH decline and high temperature (Penny, 1969; Penny, 1977; Honikel *et al.*, 1981; Offer, 1991; van Lack and Solomon, 1994; den Hertog-Meischke *et al.*, 1997; Warner *et al.*, 1997). Hence, when muscles are chilled fast enough to cause rapid temperature decline (without causing cold shortening), the protein denaturation will be reduced not only by lowering of the muscle temperature but also by slowing the pH decline, since chill rate also affects the pH decline rate.

Proteolysis and Ageing Rate Measurement

Table 4.3 shows the effects of muscle ES and chill rates on the WBS on day 1 and 14, the difference in WBS (day 14 – day 1), the WBS ageing rate constant (K), the MFI on day 1 and 14, and the difference in MFI (day 14 – day 1). On day 1, there were no differences in WBS for the SM muscle between ES treatments where as for the LD muscle, 40 sec ES samples had a lower WBS than 10 sec ES samples (muscle x ES interaction, $P<0.05$). In addition, on day 14, the LD muscle had a lower WBS than the SM muscle ($P = 0.001$). However, on day 14, the LD with 40 secs ES had the lowest WBS while the SM with 10 secs ES had the highest WBS (muscle x ES interaction, $P=0.06$). The chill rate did not result in differences in WBS ($P>0.05$). The results suggest that on day 1, only the ES treatment caused differences in tenderness and that this effect appears to be greater in the LD than the SM muscles. By day 14, the LD with 40 secs ES was still the most tender and the SM with 10 secs ES was the toughest (muscle x stim interaction, $P<0.05$). The chilling treatment did not cause any significant differences in tenderness ($P=0.06$).

The extent of ageing of the muscle can be estimated by examining the change in the value of the WBS between day 1 and 14. The 10 secs ES showed a bigger decrease in WBS from day 1 to 14 than the 40 secs ES ($P<0.05$). The LD had a greater decrease in the WBS from day 1 to 14 than the SM muscles ($P<0.05$) which showed that the LD aged more than the SM muscle overall. The faster pH decline achieved from the 40 secs ES correlated with lower WBS on day 1 compared to 10 secs stimulation, but by day 14 there was no difference in WBS between the ES treatments. This suggests that faster pH decline caused earlier ageing on day 1 than slower pH decline, and hence reduced the time to ultimate tenderness. Therefore, the slower pH decline samples exhibit a greater ageing rate but this is because they started from a higher value. Surprisingly, the results showed that the extent of ageing and ageing rate was not affected by the chill treatment.

There were only 3 ageing time-points for the SM and 4 for the LD muscles. Since the WBS data points varied significantly, not all the graphs could be fitted with the exponential function to obtain the K value. Some of those data points that could fit the equation also gave large variation in the K value and hence this may account for the lack of differences in the WBS K value due to ES and chilling treatments or between the LD and SM muscles ($P>0.10$). The average WBS K values for the LD and SM muscles were 0.53 and 0.44 unit/h respectively, but the variation was relatively large with a standard error of difference of 0.52. Therefore the WBS K was not a indicator of the effects of ES and chilling treatments on the tenderisation.

Table 4.3 also shows that the MFI values of the SM and LD muscles on days 1 and 14 were not different due to ES or chilling treatments ($P>0.05$). Overall, the LD had higher MFI values on days 1 and 14 and a greater increase in MFI from day 1 to 14 than the SM ($P<0.001$). This trend is consistent with a greater decrease in WBS value from day 1 to 14 in the LD than the SM muscles and hence indicates that the LD tenderises more than the SM muscle with time post-slaughter.

The rapid pH decline associated with the 40 sec ES treatment resulted in more tender meat as indicated by WBS on day 1 than the slow pH decline, but by day 14 there was no difference due to the treatments. This result agrees with the suggestion that rapid pH decline causes accelerated rigor onset and hence early post-mortem proteolytic breakdown of muscle proteins (Boakye and Mittal, 1993; Cheah *et al.*, 1995). The WBS and MFI values did not correlate with the drip loss or the filter paper wetness measurements ($P>0.05$, Table 4.2) and hence do not support the hypothesis that the WHC of meat is influenced by accelerated tenderisation. Furthermore, the temperature decline rate was associated with significant differences in muscle softness and WHC as indicated by SE and filter paper wetness and drip loss. While the pH decline rate was associated with differences in tenderisation but not with muscle softness, hence the muscle softness and WHC are probably not affected by the same factors that affect tenderisation.

Softness Evaluation by Sensory Panel and Instron Compression

Table 4.4 shows the effect of treatments and muscle on softness evaluation scores by the sensory panel and IC values. The SE on day 1 showed that the LD and SM were firmer

with rapid chilling than slow chilling ($P<0.001$). Although the differences due to chilling treatment were greater in the LD than the SM muscle (muscle x chill rate interaction, $P<0.01$), the SM overall tended to be firmer than the LD muscles ($P=0.071$). After 14 days of ageing, the SM muscle was softer than on day 1 ($P<0.001$, statistical analysis not presented), however there were no differences in SE softness due to chill rate or ES on day 14. It is possible that ageing for 14 days caused the muscles to be at the same level of softness for all treatments. This is supported by the WBS data where there was no difference in WBS between treatments for the SM muscle on day 14.

The IC at 10 mm depth showed that the rapid chill resulted in firmer muscle than slow chill on day 1 for the LD muscle but not for the SM muscle (muscle x chill rate interaction, $P<0.05$). On day 1, there was no difference in softness due to ES in either muscle ($P>0.05$) and overall the LD was much firmer than the SM muscle ($P<0.05$). On day 14 there was no difference in softness due to the treatments and the softness of the SM muscle on day 14 was not different to day 1 ($P>0.05$, statistical analysis not presented). For the SM, the SE of softness did not correlate with the IC ($P>0.05$) but for the LD, SE of softness did correlate with the IC ($P<0.01$, $r = 0.79$). Furthermore, the IC on day 1 showed that the SM muscle was softer ($P<0.01$) than the LD while the SE on day 1 showed the opposite ($P = 0.071$, Table 4.4). It was obvious during the softness evaluation that the LD was softer than the SM muscles. This discrepancy between SE and the IC was due to the problem of the LD muscles collapsing during the IC which gave false high compression loads as discussed in more detail in Chapter 2.2.2.

Correlation of Softness Evaluation with pH and Temperature Decline Rate Constants (pH K & Temp K)

Table 4.5 shows the Pearson correlation coefficients of muscle softness with the pH and temperature decline rate constants, pH K and Temp K. There was no correlation ($P>0.05$) between the pH K and the softness of the LD and SM muscles as measured by the IC or the SE. These results were consistent with the ANOVA of softness evaluation showing no significant effects of ES (Table 4.4). However, the Temp K was correlated ($r = 0.67$, $P<0.01$) with the SE of softness of the whole LD muscle on day 1.

The ES applied to the carcass caused differences in the pH decline rate in both the LD and SM muscles but this did not result in differences in muscle softness. In contrast, the chilling treatments did result in differences in softness and the Temp K was correlated to the SM and LD muscle softness. These results suggest that chilling treatments had greater effects on softness than the pH decline rates.

Correlation of Muscle Softness with Biochemical Analyses

Table 4.6 shows that there were some low but significant correlations ($r \sim 0.35$, $P<0.05$) between the biochemical analyses and the softness of the LD and SM muscles. The LD muscle got softer as the drip loss increased which suggests that the loss of water tends to lead to softer muscles. The SM data did not support this result as the correlation between drip loss and muscle softness was not significant ($P>0.05$). The protein solubility was also correlated with the softness of the SM muscle. It is difficult to say whether these correlations are random since there are no consistent trends and the two muscles did not exhibit similar results.

Table 4.6 also shows the correlation of pH K and Temp K with biochemical analyses. There was a high correlation between pH K and filter paper wetness value ($r = 0.59$, $P<0.05$) for the LD but not for the SM muscle. The pH K of the SM muscle showed a lower but significant correlation with drip loss ($r = 0.34$, $P<0.05$). These two tests confirm that the faster the pH decline, the greater the surface exudate and the higher the drip loss. There were also correlations between the Temp K and drip loss and the myofibrillar ATPase for the SM muscle ($P<0.05$, $r = -0.44$, 0.43 respectively). These indicate that faster temperature decline yields lower drip loss and lower myofibrillar protein denaturation.

The relationship between proteolysis and protein denaturation with muscle softness is unclear since there is a lack of correlation between the tenderness and protein denaturation data with muscle softness. Muscle softness was influenced by the temperature decline rate while the protein denaturation was influenced by both temperature and pH decline rates and tenderisation appeared to be influenced mainly by the pH decline rate. This suggests that muscle softness is more affected by protein denaturation than proteolysis. That is, greater protein denaturation rather than rapid tenderisation would lead to softer muscles.

The Effects of Muscle Fibre Type on WHC and Softness

Muscle fibre types play an important role in muscle characteristics that could potentially affect the biochemical properties, the WHC and the softness of meat. The results have shown that even though the SM had smaller differences in the pH and temperature decline rates (pH K and Temp K) than the LD muscle, the SM had greater protein denaturation and subsequently lower WHC. This could be due to the SM muscle having more white muscle fibre than the LD as stated by (Hunt and Hedrick, 1977; Devine *et al.*, 1984). In addition, muscles composed of predominantly white muscle fibres are considered to tenderise more rapidly than muscles composed of predominantly red fibre type because they have more proteolytic enzymes (Honikel *et al.*, 1981; Boakye and Mittal, 1993; Cheah *et al.*, 1995), but this observation has not been supported by this experiment. Instead, the SM aged more slowly and to a lesser extent than the LD muscle. A contributing factor to this observed difference in ageing could be due to the fact that the SM has much smaller differences in pH and temperature decline rates than the LD muscle in this experiment. Previous researchers have found that the SM had much faster pH decline than the LD muscle (Hunt and Hedrick, 1977; den Hertog-Meischke *et al.*, 1997). One of the reasons that the SM tenderises less than the LD muscle could be due to greater amount of connective tissue in the SM than in the LD muscle.

The SM had greater protein denaturation and lower WHC, and hence would be expected to be softer than the LD muscles, but the SM was instead firmer than the LD muscle as assessed by SE on day 1. Perhaps due to its shape, the amount of connective tissue and physical structure (i.e. muscle fibre orientation and size of fibre bundles), the SM is naturally firmer than the LD muscle and hence this would mask the effects of protein denaturation and poor WHC on muscle softness. As a result, the effects of muscle fibre type on softness could not be determined from the SM and LD muscles, unless they had similar physical structure.

The Relationship Between WHC and Muscle Softness

There was a negative correlation between the drip loss and the softness of the LD muscle as assessed by the SE. This suggests that the higher the drip loss, the softer the muscle. However, the lack of correlation with other WHC measurements (filter paper wetness, purge and cook loss) with muscle softness failed to substantiate this relationship. One likely reason that could explain the lack of correlation between the WHC and muscle

softness is that the softness assessment methods were not well developed. The sensory panel was not trained in the assessment of muscle softness and the IC method was still in the developmental stage and hence exhibited large variation in the softness measurement results. As a result the IC could not detect differences in softness due to the treatments. Furthermore, the IC results showed that the LD was firmer than the SM muscle while the SE showed that the SM was instead firmer than the LD muscle. As a result, there was very little correlation between the WHC and the muscle softness measurements from this experiment to show that the WHC has a direct effect on muscle softness. However, due to poor results from the softness measurement, the relationship between WHC and muscle softness can not be discounted by this study. Furthermore, the WHC and muscle softness measurements have shown differences due to chilling treatment and not the stimulation. Therefore it indicates that the WHC and muscle softness are both affected by a common factor, and that is the temperature decline rate.

4.4 Conclusion

The combination of ES and chill rates resulted in differences in pH and temperature decline rates in both muscles, but the LD had much greater differences than the SM muscle. Consequently, the proteolysis and protein denaturation measurements reflected this. The MFI and WBS measurements showed that the LD muscle aged faster and to a greater extent than the SM muscle. However, on average the SM had greater drip loss and filter paper wetness but lower myofibrillar ATPase activity and total protein solubility than the LD muscle. This showed that the SM was more susceptible to denaturation and hence poorer WHC than the LD muscle.

It was found that the pH and temperature decline rates both had effects on the WHC and protein denaturation. The combination of faster pH decline with slower temperature decline resulted in increased drip loss and filter paper wetness and lower myofibrillar ATPase activity. These results were supported with correlations between pH K and Temp K with the drip loss, filter paper wetness and protein denaturation measurements. Since higher protein denaturation coincided with higher drip loss and filter paper wetness, it could be concluded that the WHC is closely influenced by protein denaturation.

This experiment showed very little correlation between muscle softness and proteolysis and hence did not support the hypothesis that rapid tenderisation causes rapid softening of muscles. It was found that muscle softness was mainly affected by temperature decline rate while the protein denaturation was affected by both temperature and pH decline rates and proteolysis was mainly affected by the pH decline rate. This suggests that muscle softness is more influenced by protein denaturation rather than proteolysis. That is, higher protein denaturation and lower WHC would lead to softer muscles.

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Table 4.1: The effects of electrical stimulation (10s ES vs 40s ES) and chilling rates (RC vs SC) on the pH (pH K) and temperature decline rate (Temp K) (values in parentheses are the transformed anti-log values), ultimate pH, colour readings (L*, a*) and sarcomere length. Each value is a least squares mean.

		10s ES[1]		40s ES[2]			P value						
	muscle	RC[3]	SC[4]	RC	SC	SED[5]	M[6]	E[7]	C[8]	MxE	MxC	CxE	MxCxE
Log pH K (pH K)[10]	LD	-0.87 (0.13)	-0.59 (0.26)	-0.03 (0.94)	-0.04 (0.91)	0.14	0.001	0.001	NS[9]	0.002	NS	0.062	NS
	SM	-1.04 (0.09)	-0.86 (0.14)	-0.67 (0.21)	-0.70 (0.20)								
Log Temp K (Temp K)[10]	LD	-0.44 (0.36)	-0.84 (0.14)	-0.43 (0.37)	-0.79 (0.16)	0.05	0.001	NS	0.001	NS	0.001	NS	NS
	SM	-0.94 (0.11)	-1.26 (0.05)	-0.93 (0.12)	-1.26 (0.05)								
Ultimate pH	LD	5.54	5.47	5.53	5.49	0.03	NS	NS	0.038	NS	NS	NS	NS
	SM	5.53	5.50	5.50	5.50								
L*	LD	38.90	40.70	39.46	40.26	0.80	NS	NS	0.003	NS	NS	NS	NS
	SM	38.85	39.66	38.65	40.31								
a*	LD	8.04	10.42	8.45	8.67	4.3	0.057	NS	0.009	NS	NS	NS	0.070
	SM	8.60	10.68	10.48	12.34								
Sarcomere length (μm)	LD	1.85	1.90	1.92	1.88	0.05	NS	NS	NS	NS	NS	NS	NS
	SM	1.84	1.84	1.83	1.82								

[1]10s ES = 10 secs electrical stimulation (45 V & 500 mA), [2]40s ES = 40 secs electrical stimulation (45 V & 500 mA)
[3]RC = Rapid Chill (1 °C for 10 h), [4]SC = Slow Chill (18 °C for 2 h and then at 6 °C for 8 h), [5]SED = standard error of difference
[6]M = muscle, [7]E = electrical stimulation, [8]C = chill rate, [9]NS = not significant, P>0.10
[10]pH K = anti $\log_{10}$ pH K after transformation of Log pH K

Table 4.2: The effects of muscle electrical stimulation and chilling rates on the WHC and protein denaturation as measured by drip loss, purge at 14 days post-mortem, filter paper wetness, myofibrillar Ca^{2+}-ATPase activity, sarcoplasmic and total protein solubility. Each value is a least squares mean.

		10s ES[1]		40s ES[2]			P value						
	muscle	RC[3]	SC[4]	RC	SC	SED[5]	M[6]	S[7]	C[8]	MxE	MxC	CxE	MxCxE
Drip loss (%)	LD	0.98	1.45	1.27	1.42	0.24	0.001	NS[9]	0.014	NS	NS	NS	0.086
	SM	2.06	2.10	2.07	2.61								
Purge - day 14 (%)	SM	3.22	3.84	3.37	3.63	0.39	NS	NS	NS	NS	NS	NS	NS
Filter paper wetness (mg/cm^2)	LD	1.15	1.86	1.79	2.28	0.27	0.001	0.056	0.001	0.033	NS	NS	NS
	SM	3.91	4.62	3.69	4.05								
Myofibrillar ATPase activity (μmol Pi/mg/min)	LD	0.62	0.63	0.60	0.49	37	0.058	0.001	0.041	NS	NS	NS	0.088
	SM	0.59	0.56	0.54	0.51								
Sarcoplasmic protein solubility (mg/g)	LD	44.6	44.7	44.2	42.5	3.0	NS	NS	NS	NS	NS	NS	NS
	SM	43.5	42.8	42.5	42.2								
Total protein solubility (mg/g)	LD	85.1	86.6	83.5	84.0	5.5	0.039	NS	NS	NS	NS	NS	NS
	SM	78.9	82.3	79.2	76.5								

[1]10s ES = 10 secs electrical stimulation (45 V & 500 mA), [2]40s ES = 40 secs electrical stimulation (45 V & 500 mA)

[3]RC = Rapid Chill (1 °C for 10 h), [4]SC = Slow Chill (18 °C for 2 h and then at 6 °C for 8 h), [5]SED = standard error of difference

[6]M = muscle, [7]E = electrical stimulation, [8]C = chill rate, [9]NS = not significant, P>0.10

Table 4.3: The effects of muscle electrical stimulation and chilling rates on proteolysis as measured by the Warner-Bratzler shear (WBS) on day 1 and 14 post-mortem, difference in WBS (day 14 – day 1), WBS ageing rate constant (K), myofibrillar fragmentation index (MFI) day 1 and 14, and difference in MFI (day 14 – day 1). Each value is a least squares mean.

Proteolysis	muscle	10s ES[1]		40s ES[2]		SED[5]	P value						
		RC[3]	SC[4]	RC	SC		M[6]	E[7]	C[8]	MxE	MxC	CxE	MxCxE
WBS day 1 (Kg)	LD	7.21	6.42	5.28	5.19	0.60	NS	0.003	NS[9]	0.027	NS	NS	NS
	SM	5.99	5.68	5.44	5.76								
WBS day14 (Kg)	LD	4.63	3.69	3.82	3.77	0.33	0.001	NS	0.09	0.060	NS	0.07	NS
	SM	4.76	4.56	4.87	4.95								
Difference in WBS (Kg)	LD	-2.58	-2.73	-1.46	-1.42	0.64	0.001	0.01	NS	NS	NS	NS	NS
	SM	-1.24	-1.12	-0.58	-0.98								
WBS ageing rate constant (K)	LD	0.54	0.76	0.31	0.52	0.52	NS	NS	NS	NS	NS	NS	NS
	SM	0.89	0.31	0.27	0.30								
MFI day 1	LD	59	69	63	66	5.8	0.001	NS	NS	NS	NS	NS	NS
	SM	48	48	49	43								
MFI day 14	LD	77	78	77	74	3.8	0.001	0.076	NS	NS	NS	NS	NS
	SM	55	51	50	47								
Difference in MFI	LD	16.5	9.0	12.9	6.5	6.7	0.001	NS	NS	NS	0.071	NS	NS
	SM	-0.2	1.5	-4.0	3.7								

[1]10s ES = 10 secs electrical stimulation (45 V & 500 mA), [2]40s ES = 40 secs electrical stimulation (45 V & 500 mA)

[3]RC = Rapid Chill (1 °C for 10 h), [4]SC = Slow Chill (18 °C for 2 h and then at 6 °C for 8 h), [5]SED = standard error of difference

[6]M = muscle, [7]E = electrical stimulation, [8]C = chill rate, [9]NS = not significant, P>0.10

Table 4.4: The effects of muscle electrical stimulation and chilling rates on the softness of *Semimembranosus* (SM) and *Longissimus dorsi* muscles on day 1 and on the SM muscle on day 14 as measured by the sensory evaluation and the Instron compression (IC) load at 10 mm compression depth. Each value is a least squares mean.

Softness Evaluation	muscle	10s ES[1]		40s ES[2]		SED[5]	P value						
		RC[3]	SC[4]	RC	SC		M[6]	E[7]	C[8]	MxE	MxC	CxE	MxCxE
Sensory Evaluation[10] Day 1	LD	4.89	3.59	5.19	3.89								
	SM	4.89	4.49	4.82	4.60	0.05	0.071	0.071	0.001	0.098	0.002	NS[9]	NS
Sensory Evaluation[11] Day 14	SM	3.86	3.82	4.00	3.77	0.40	-	NS	NS	-	-	NS	-
IC - Day 1 (Kg)	LD	0.55	0.39	0.60	0.47								
	SM	0.21	0.22	0.21	0.27	0.09	0.001	NS	NS	NS	0.040	NS	NS
IC - Day 14 (Kg)	SM	0.24	0.24	0.23	0.26	0.04	-	NS	NS	-	-	NS	-

[1]10s ES = 10 secs electrical stimulation (45 V & 500 mA), [2]40s ES = 40 secs electrical stimulation (45 V & 500 mA)

[3]RC = Rapid Chill (1 °C for 10 h), [4]SC = Slow Chill (18 °C for 2 h and then at 6 °C for 8 h), [5]SED = standard error of difference

[6]M = muscle, [7]E = electrical stimulation, [8]C = chill rate, [9]NS = not significant, P>0.10, [10]softness score assessed on whole muscle, [11]softness score assessed on muscle blocks; 1 = extremely soft, 8 = extremely firm

Table 4.5: Pearson correlation coefficients (r) with their respective P values (P) for the softness evaluation by the Instron compression (IC) load at 10 mm compression depths and by sensory evaluation with the pH and temperature decline rate constants (pH K and Temp K) for the *Longissimus dorsi* (LD) and *Semimembranosus* muscles for days 1 and 14 post-mortem.

	pH K				**Temp K**			
	LD		SM		LD		SM	
Softness Evaluation	r	P	r	P	r	P	r	P
IC - 10 mm Day 1	0.002	0.99	0.27	0.10	0.25	0.18	-0.28	0.09
Sensory Evaluation Day 1	0.14[a]	0.39	-0.05[a]	0.75	**0.67[a]**	**0.001**	**0.55[b]**	**0.005**
Sensory Evaluation Day 14			-0.02[b]	0.92			0.07[b]	0.69

[a] = correlation using whole muscle, n = 40

[b] = correlation using muscle block, n = 40

Table 4.6: Pearson correlation coefficients (r) of various biochemical and physical measurements of the *Longissimus dorsi* (LD) and *Semimembranosus* muscles with muscle softness on day 1 as measured by Instron compression (IC) load at 10 mm compression depth and sensory evaluation (SE) and the pH and temperature decline rate constants (pH K and Temp K).

	SE - day 1		IC – Day 1		pH K		Temp K	
	LD	SM	LD	SM	LD	SM	LD	SM
Sarcomere length	-0.19	-0.12	-0.12	0.01	0.18	-0.10	-0.25	-0.05
N[1]	40	40	35	40	40	38	36	39
Drip loss	**-0.34***	-0.04	-0.10	0.08	0.13	**0.34***	-0.15	**-0.44***
N	40	40	35	40	40	38	36	39
Filter paper wetness	-0.33	-0.06	-0.27	0.10	**0.59****	-0.23	-0.21	-0.25
N	24	24	24	24	24	22	21	24
Ca^{2+}-ATPase	-0.14	-0.01	-0.14	-0.20	-0.25	-0.23	-0.02	**0.43***
N	39	40	34	40	39	38	35	39
Sarcoplasmic protein solubility	0.13	**0.37***	0.30	**0.32***	0.11	0.24	-0.20	-0.19
N	40	40	35	40	40	38	36	39
Total protein solubility	0.20	0.25	0.21	**0.38***	-0.08	0.09	0.04	-0.24
N	38	40	33	40	38	38	34	39
MFI[2] day 1	-0.26	0.04	-0.24	-0.31	0.21	-0.14	-0.29	0.13
N	32	32	27	32	32	30	28	32
MFI day 14	0.02	0.24	0.13	0.18	-0.03	0.15	-0.19	0.03
N	40	32	35	32	40	31	36	31
WBS[3] K	-0.31	-0.06	-0.29	-0.20	**-0.39***	-0.35	-0.35	-0.04
N	30	9	25	9	30	9	27	9

* Correlation coefficient is significant at $P<0.05$

[1]N = number of samples, [2]MFI = Myofibrillar fragmentation index, [3]WBS K = Warner-Bratzler shear ageing rate constant

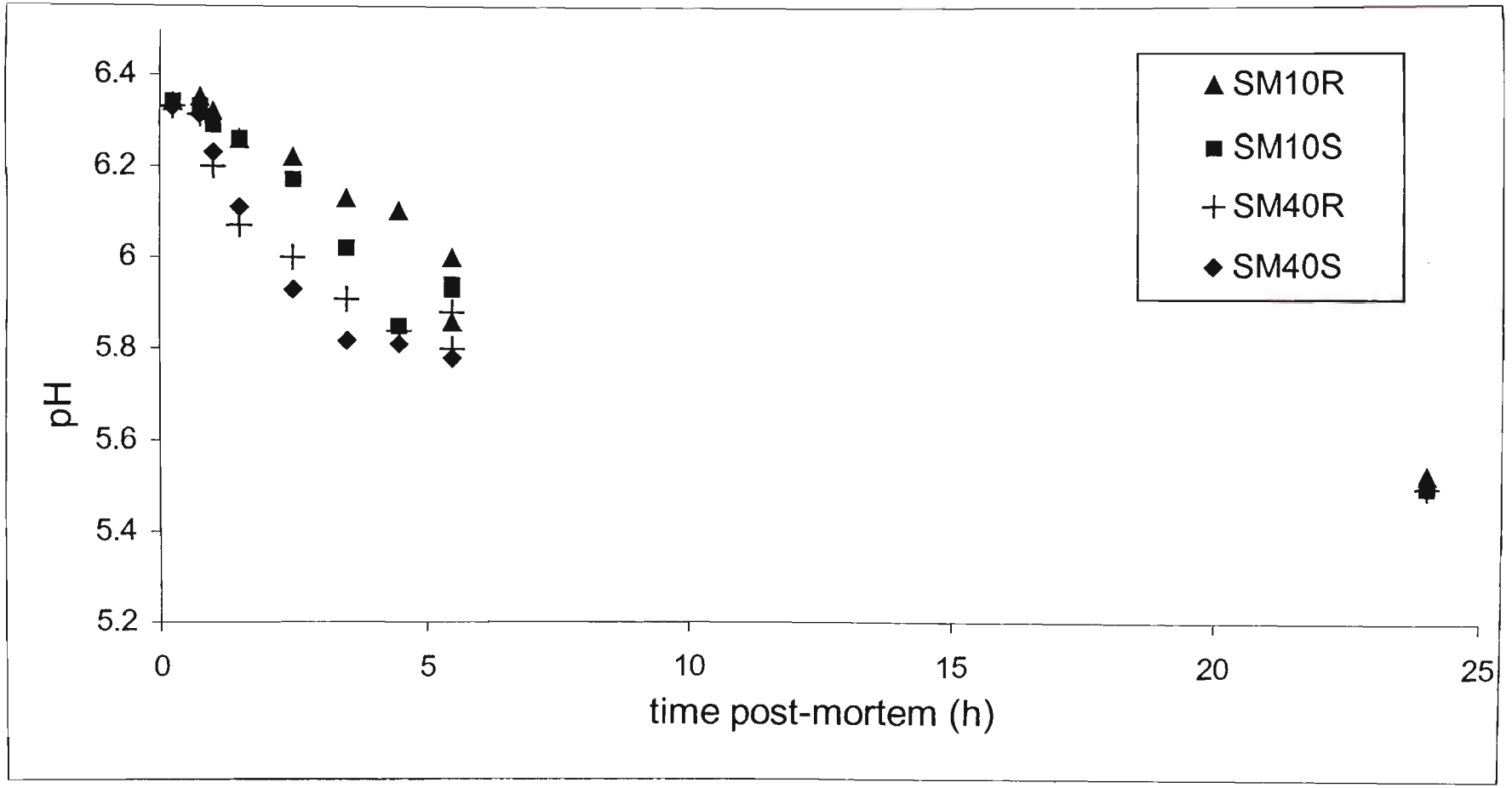

Figure 4.1: The effects of muscle electrical stimulation and chilling rates on the pH decline rate of beef SM muscles.

SM10R = SM muscle with 10 secs stimulation (45 V & 500 mA) and rapid chill (1 °C for 10 h), SM40R = SM muscle with 40 secs stimulation and rapid chill.
SM10S = SM muscle with 10 secs stimulation and slow chill (18 °C for 2 h and then at 6 °C for 8 h), SM40S = SM muscle with 40 secs stimulation and slow chill.

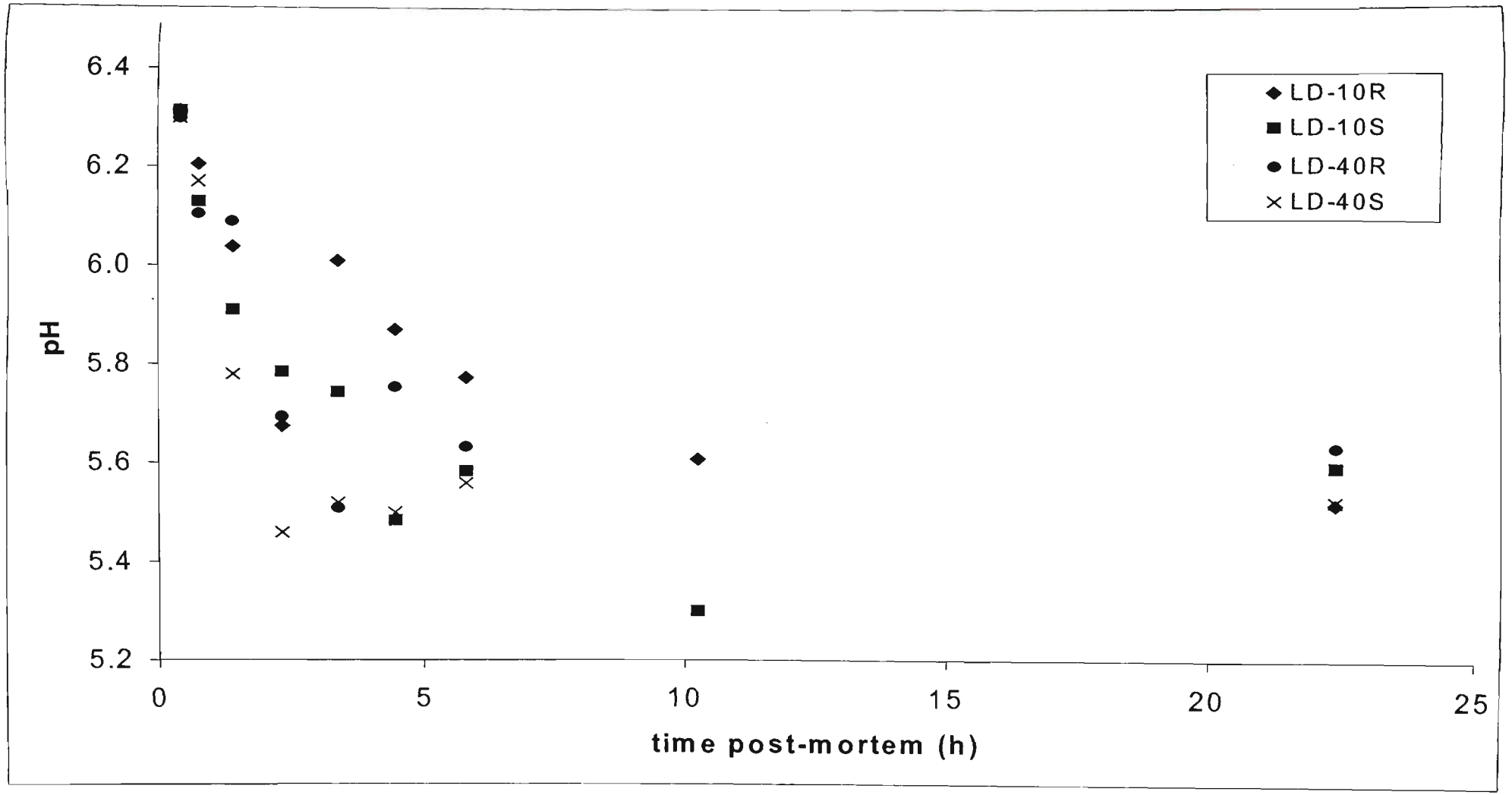

Figure 4.2: The effects of muscle electrical stimulation and chilling rates on the pH decline rate of beef *Longissimus dorsi* muscles.

LD10R = LD muscle with 10 secs stimulation (45 V & 500 mA) and rapid chill (1 °C for 10 h), LD40R = LD muscle with 40 secs stimulation and rapid chill.
LD10S = LD muscle with 10 secs stimulation and slow chill (18 °C for 2 h and then at 6 °C for 8 h), LD40S = LD muscle with 40 secs stimulation and slow chill.

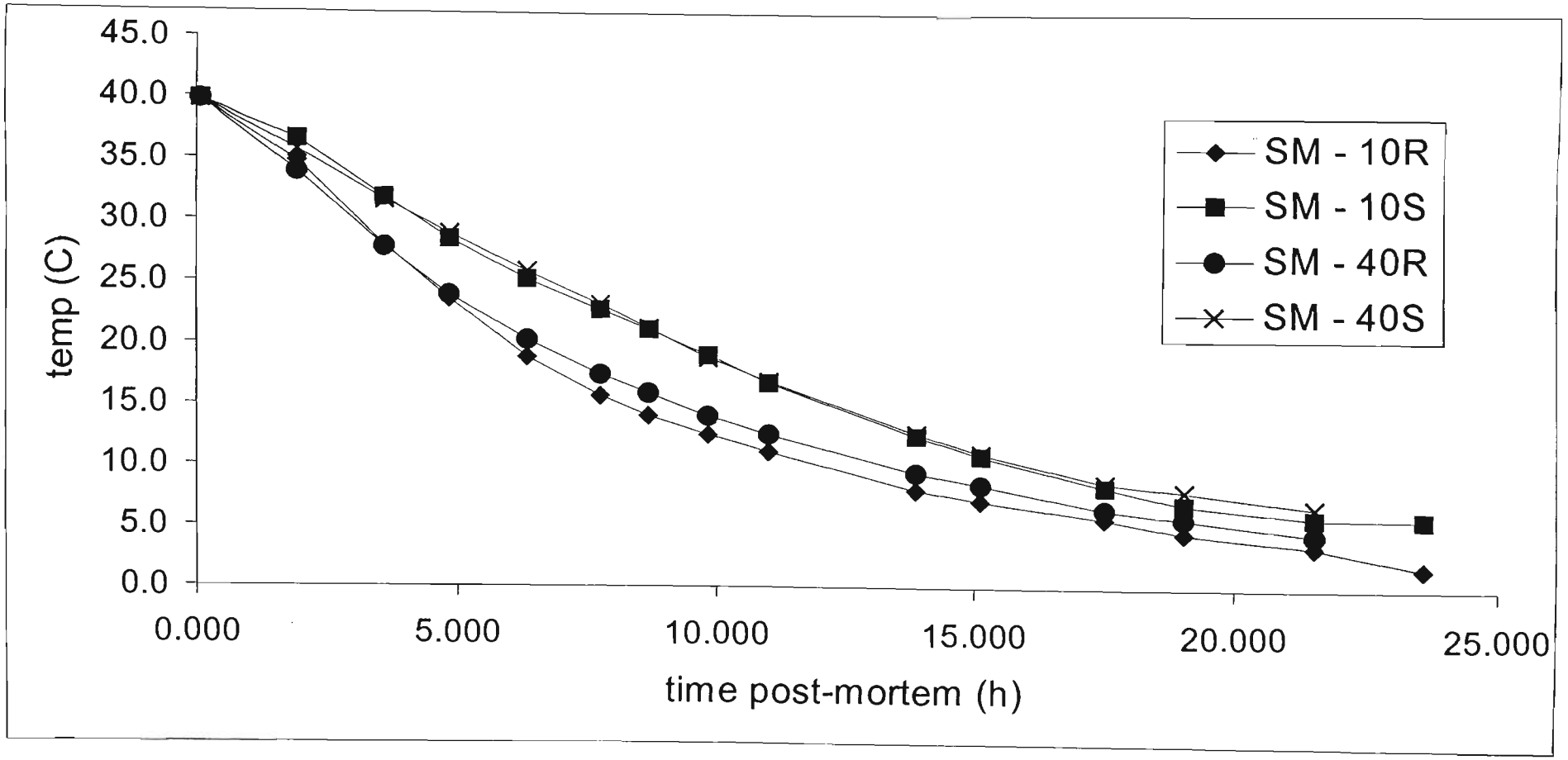

Figure 4.3: The effects of muscle electrical stimulation and chilling rates on the temperature decline rate of beef *Semimembranosus* muscles.

SM10R = SM muscle with 10 secs stimulation (45 V & 500 mA) and rapid chill (1 °C for 10 h), SM40R = SM muscle with 40 secs stimulation and rapid chill.
SM10S = SM muscle with 10 secs stimulation and slow chill (18 °C for 2 h and then at 6 °C for 8 h), SM40S = SM muscle with 40 secs stimulation and slow chill.

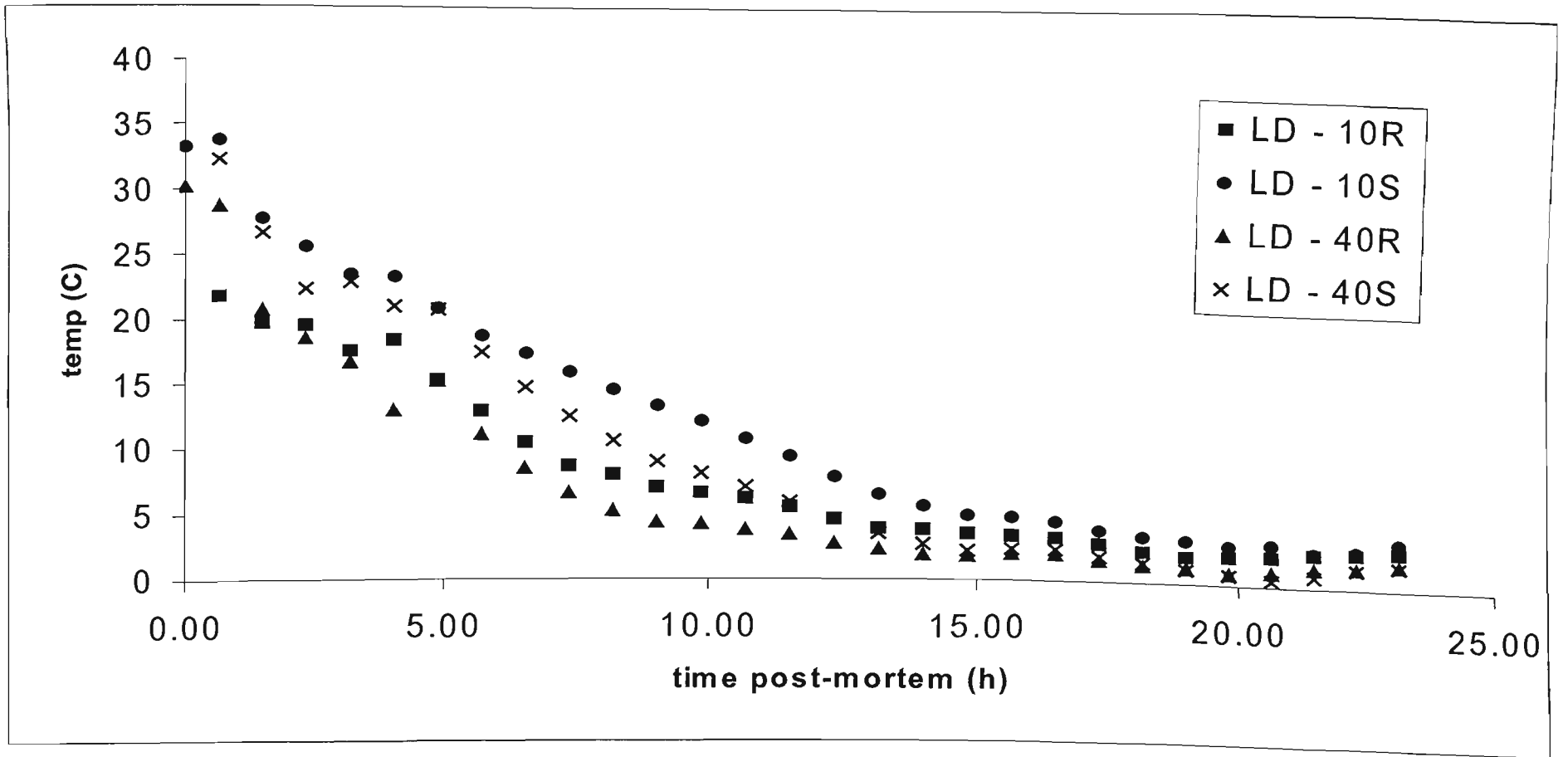

Figure 4.4: The effects of muscle electrical stimulation and chilling rates on the temperature decline rate of beef *Longissimus dorsi* muscles.

LD10R = LD muscle with 10 secs stimulation (45 V & 500 mA) and rapid chill (1 °C for 10 h), LD40R = LD muscle with 40 secs stimulation and rapid chill.
LD10S = LD muscle with 10 secs stimulation and slow chill (18 °C for 2 h and then at 6 °C for 8 h), LD40S = LD muscle with 40 secs stimulation and slow chill.

5 GENERAL CONCLUSION

The MFI of meat was affected by the homogenisation speed and time, especially for unaged samples. With increasing speed and time of homogenisation, the difference between unaged and aged meat decreases. The optimum method that sufficiently homogenised both aged and unaged samples and at the same time resulted in the greatest difference between aged and unaged samples was low speed for 30 secs. This method was used for investigating muscle tenderisation in subsequent chapters. The MFI of frozen samples was found to be significantly lower than that of fresh samples for both aged and unaged meat. However, the difference in MFI between day 1 and day 14 of the frozen was the same as the fresh samples. Therefore, frozen meat could be used for MFI analysis but the MFI values of the frozen samples could not be compared directly with the fresh samples.

When very soft muscles were positioned upright for the IC parallel to the muscle fibre direction, the muscles tended to collapse out of shape which made the samples more compact. This made the soft samples appear firmer than the samples that did not collapse, hence the IC was limited by this problem. The problem was overcome by two methods. One method was to cut the muscles with a cylindrical corer and then glue one end of each core to the compression head to prevent the soft samples from collapsing. The compression of the meat cores was parallel to the muscle fibre direction (or close to being parallel to the fibres in the case of the LD). The other method was to compress meat blocks across the muscle fibre direction

The result obtained from the cylindrical cores correlated with the SE of softness but the two methods were not able to detect differences in muscle softness between the high and medium temperature treatments. In contrast, when samples were compressed perpendicular to the muscle fibre direction on muscle blocks, differences in softness due to temperature treatment were detected. However, this method did not correlate to the SE scores. This suggested that the judges emphasised the assessment of softness parallel to the muscle fibre direction, which is the softest direction. The IC at 5 mm depth perpendicular to the muscle fibre was the preferred method even though it did not correlate to the SE since it could differentiate muscle softness due to treatments.

The WHC of meat was influenced by the temperature and pH decline rates and the proportion of different fibre types in a muscle. Rapid pH decline and high temperature increased protein denaturation and reduced WHC. Even though the LD had much faster pH decline than the SM, the SM muscle with higher proportion of type IIb fibres suffered more water loss and protein denaturation than the LD muscle which contains less type IIb fibres.

A degree of accelerated tenderisation due to pH decline was observed in Chapter 4 which corresponded to softer muscles on day 1. The results suggested that accelerated tenderisation may have contributed to rapid softening of muscles post-mortem. It was also found that day 14 samples were softer than day 1 samples, hence ageing did result in softening of muscles. However, accelerated tenderisation was not observed in Chapter 3 to support the hypothesis that accelerated tenderisation may cause rapid softening of muscles even though the high temperature treatment resulted in rapid pH decline rate. Furthermore, the sarcomere length in both Chapters 3 and 4 showed minimal shortening and was not different due to treatments. Hence the sarcomere length was not considered to have had a major influence on the WHC or softness.

The protein denaturation and water loss were both higher for samples with slow chill rate. There were some correlations between protein denaturation and WHC with muscle softness, hence, there was evidence to support the hypothesis that increasing water loss leads to increasing muscle softness. Accelerated tenderisation early post rigor is desirable in terms of meat tenderness. However, the conditions that cause this also cause more water loss from the muscle and increases softness, which is undesirable. Therefore the industry recommendation is a balance between tenderness and WHC to obtain high quality beef.