Post Mortem Carcass Interventions to Improve Beef Quality

by

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Declaration

By submitting this thesis electronically, I declare that entirety of the work contained therein is my own, original work and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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Abstract

A total of 32 cattle were divided into four groups of eight each (A1-8, A9-16, A17-24, B1-B8) to be slaughtered on different days over a period of 45 days. All the cattle in Groups A were of Bonsmara type and those in Group B were of Charolais type. Three treatments, Tenderstretch (TS), Tendercut (TC) and Hock suspension (HS) were randomly allocated to each of the 48 sides from group A. Only two treatments (TC and HS) were implemented on the carcass sides in Group B alternating between the right and left sides. Two muscles from each side namely *Gluteus medius* and *Longissimus dorsi* were evaluated for purge, cooking loss, shear force and sarcomere length after 2, 4, 6, 10 and 14 days of aging. Paired t-Tests were performed for each pair of treatments and each day separately, on all variables accessed (Snedecor, 1980). The differences in purge and cooking loss between treatments were all found to be inconclusive for each day of aging. Although purge had significant differences between the treatments TC and TS for the GM and LD muscle after 14 days of aging (P = 0.0341 and P = 0.0348 respectively) these were found to be open to doubt as the treatment that delivers the most purge differs between muscles and that the two treatments delivered no differences compared to its HS values. Aging had a significant effect on purge as it doubles after 14 days of aging. Cooking loss values only differed significantly on day 2 for the LD muscle between treatments TC and HS. The differences in shear force were all smaller than 0.3205 kg/ 1.27cm and not consistent over all carcasses. A mean positive improvement in tenderness was calculated from high difference in mean values from some carcasses although some carcasses showed a decrease in tenderness when using TS and TC, which suggests that the treatments are of no relevance towards the industry. Although the differences in shear force become smaller as aging commences, it is not constant, a phenomenon most probably due to the variance between animals. Aging again had the most significant effect (P<.0001) on shear force. Correlations between sarcomere lengths and shear force were low for all the treatments on the GM muscle (HS = -0.453; TC = -0.401 and TS = -0.2) but in the LD muscle the TS method showed a higher correlation (TS = -0.665) than the other treatments (HS = 0.059 and TC = 0.059).

Uittreksel

`n Totaal van 32 beeste was opgedeel in vier groepe van agt elk (A1-8, A9-16, A17-24, B1-B8) wat geslag is op verskillende dae oor n periode van 45 dae. Beeste van die A groep was almal Bonsmara tipe en die van die B groep charolais tipe. Drie behandelings naamlik Tenderstretch (TS), Tendercut (TC) en Hak suspensie (HS) was ewekansig tot die 48 sye van die karkasse van groep A toegedeel. Groep B se 16 sye is net met TC en HS ewekansig tot die linker en regter sy toegedeel. Twee spiere naamlik *Gluteus medius* en *Longissimus dorsi* was geevalueer vir drup verlies, kook verlies, sarkomeer lengte en taaihied na 2, 4, 6, 10 en 14 dae se veroudering. Gepaarde t – toetse is gedoen vir elke paar behandelings vir elke dag van veroudering op al die veranderlikes genoem. Die verskil in drup verlies en kook verlies tussen behandelings was as nie betekeinisvol bestempel. Behalwe vir die feit dat drup verlies betekenisvolle verskille getoon het tussen die behandelings TC en TS vir die GM ($P = 0.0341$) en die LD ($P = 0.0348$) spiere na 14 dae se veroudering was dit bevind as nie betekenisvol juis oor dat die twee behandelings teenoor hul HS waardes geen verskille getoon het nie. Veroudering van die vleis het wel die grootste betekenisvolle verskil in drupverlies gemaak waar dit amper verdubbel soos die vleis verouder vir 14 dae. Kookverlies het net op dag twee n betekenisvolle verskil getoon in die LD spier vir die HS – TC kombinasie. Die verskil in taaiheid was almal kleiner as 0.3205 kg/ 1.27cm en nie kostant vir alle karkasse nie. n' Positiewe gemiddelde verbetering in sagtheid is verkry deur die kalkulasie van hoë positiewe waardes en lae negatiewe waardes vir sommmige karkasse wat n laer sagtheid getoon het wanneer TS en TC gebruik is. Hierdie onkonsekwente verbeterings in sagtheid maak dat hierdie behandelings van min praktiese nut vir die bedryf is. Alhoewel hierdie verskille tussen behandelings kleiner raak tydens veroudering, is dit nie konstant nie, wat as gevolg van die variasies tussen diere kan wees. Veroudering het weereens die mees betekenisvolle effek op die vleis getoon ($P< 0.001$). Die korrelasie tussen sarkomeer lengte en WBSF taaiheid was laag vir alle behandelings in die GM spier (HS = -0.453; TC = -0.401 en TS = -0.2) behalwe vir die LD spier waar die TS behandeling n hoer korrelasie van TS = -0.665 as die ander twee behandelings (HS = 0.059 en TC = 0.059) opgelewer het.

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implement Total Quality Management-like systems to improve the quality and consistency of beef tenderness (Tatum *et al,* 1999).

Chapter 2

Muscle tenderness

According to a consumer survey performed by the Food Marketing Institute (1988, 1998) as cited by Tatum *et al,* 1999, the most important driver of food purchase decisions every year is “taste.” The NLSMB (1995) researched consumers’ perceptions of “taste” and found that it was strongly correlated to differences in juiciness (r = 0.79), flavour (r = 0.86) and tenderness (r = 0.85) (Tatum *et al,* 1999).

Juiciness is determined by the total amount of water and fat still remaining in the muscle after it has been cooked. The level of juiciness is influenced by the degree of doneness (Lorenzen *et al,* 1999), the water holding capacity (WHC), being high for juicy and low for less juicy, and the amount of intramuscular fat of the meat (Tatum *et al,* 1999).

Beef flavour is a result of the amount and composition of intramuscular fat which is affected by the type of feed, being forage or grain fed () and by the number of days the cattle are fed a high concentrate finishing diet (Smith, 1997 Bowling *et al,* 1978 Dolezal *et al,* 1982 as cited by Tatum *et al,* 1999).

Beef tenderness can be described with five contributing factors which includes the amount of connective tissue within the muscle, the amount of collagen cross-linkages formed in the connective tissue, the contractile state of the myofibrils during rigor mortis, the amount and distribution of intramuscular marbling and the extent of post-mortem muscle proteolysis during the aging process (Smith, 1997 as cited by Tatum *et al,* 1999).

Koohmaraie, Kent, Shackleford, Veiseth and Wheeler (2002) described muscle tenderness as a function of three major components namely the amount and composition of connective tissue, sarcomere length and the degree of proteolysis of the myofibrillar proteins. Every one of these factors involvement in the eventual tenderness level of the meat will differ between muscle, animal, pre- and post slaughter factors, the *post-mortem* aging length and the temperature at which it is kept (As cited by Thompson *et al,* 2006).

2.1 Connective tissue

Collagen, which forms the main component of connective tissue, is well known for its influence on the toughness of beef and its contribution to “background” toughness (Bailey & Light, 1989). Ouali, Demeyer

and Raichon, (1992) and Sentandreu, Coulis and Ouali, (2002) again stressed the fact that “background” toughness is a function of the amount of connective tissue and the chemical composition thereof coinciding with the age at which the animal is slaughtered. Collagen, therefore, is the main factor determining the texture of the meat, obtained in the absence of any myofibrillar shortening, however it is said that the slight texture variations between muscles is not a result of the amount of collagen but rather the quality thereof (Bailey & Light, 1989 as cited by Torrescano *et al,* 2003).

Although a large correlation was found between total collagen content and muscle toughness (Dransfield, 1977), Cross, Carpenter and Smith (1973) significantly related collagen solubility to the contribution of connective tissue toughness and its influence in the slight variations in texture between different muscles throughout different locations in the carcass (As cited by Torrescano *et al,* 2003). Bailey & Light (1989) furthermore demonstrated that it is the collagen solubility that decreases during the process of aging and weight gain and not the amount of collagen present within the muscle which coincides with decreased tenderness. Torrescano *et al,* (2003) measured shear force values of 14 different bovine muscles and documented their relation to muscle connective tissue characteristics. The lowest collagen content was found for the *Psoas major* PM muscle whereas the highest collagen content was seen in the *Flexor digitorum* (FD) muscle. Other reports from Cross *et al,* 1973 showed a lower total collagen content for the *Longissimus dorsi* (LD) muscle than for the *Biceps femoris* (BF) and *Semitendinosus* (ST) muscles (Torrescano *et al,* 2003).

A difference ($P<0.05$) between muscles were found for the total soluble collagen content (Torrescano *et al,* 2003). Various studies found the lowest soluble collagen content in the ST, *Semimembranosus* (SM) and BF muscles whereas the highest amount was reported in the *Psoas major* (PM) muscle (Seideman, (1986) as cited by Torrescano *et al,* (2003). The amount and solubility of collagen has been found to be strongly related to the animal diet. Young animals fed a high grain diet showed a 50% increase in collagen solubility compared to animals fed corn silage (Rompala & Jones, 1984 as cited by Torrescano *et al,* 2003). In addition, animals on a high energy diet had higher levels of total collagen and a similar amount of insoluble collagen according to Crouse, Cross & Seideman (1985) (As cited by Torrescano *et al,* 2003)

From previous studies from De Smet *et al,* 1998; Destefanis, Barge, Brugiapaglia & Tassone, 2000 , highly positive correlations were found between Warner Bratzler Shear force (WBSF) and the total collagen content ($r = 0.723$; $P<0.01$) as well as between WBSF and the insoluble collagen content ($r = 0.661$; $P<0.01$). In addition, a high positive correlation was reported by Crouse *et al,* (1985) between the total collagen and the total insoluble collagen content. (As cited by Torrescano *et al,* 2003)

Marsh and Leet (1966) however stated that the subsequent toughness of contracted muscle was more a result of the actomyosin formation within the fibres itself than the contribution of “background toughness” (As cited by Herring *et al,* 1967).

2.2 Sarcomere length

The relationship between sarcomere length and meat tenderness has caused much debate between researches. Studies from Locker *et al,* (1960); Herring *et al,* (1965a); Hostetler *et al,* (1972); Bouton *et al,* (1973); and Davis *et al,* (1979) revealed a positive correlation between sarcomere shortening and resulting toughness while others have encountered no substantial relationship between these two factors (Culler *et al,* 1978; Parrish *et al,* 1979; Smith *et al,* 1979; Seideman *et al,* 1987; Shackleford *et al,* 1994; Koohmaraie *et al,* 1995)

Locker *et al,* (1960) documented a decrease in tenderness coinciding with a decrease in sarcomere length. Correlations between sarcomere length and shear force values of previous studies have differed from between -0.34 to -0.80 which indicates a slight to heavy increase in tenderness with an increase in sarcomere length (Herring *et al,* 1965; Hostetler *et al,* 1972; Dutson *et al,* 1976; Wang *et al,* 1994). Significantly shorter sarcomere lengths were found in the *M. semimembranosus, M. Gluteus medius, M. Biceps femoris and M. Longissimus dorsi,* correlating to a higher shear force measurement for sarcomeres shorter than 2.0µm. Supporting this positive relationship between sarcomere length and meat tenderness, Marsh and Leet (1966) found a maximum toughness in pre-rigor excised meat at a 40% shortening of the sarcomere and an increased tenderness below 40% shortening (As cited by Koohmaraie 1996). Bouton *et al,* (1973) stated that some shear force values declined exponentially as the sarcomere lengths increased. All the muscles however do not demonstrate this correlation between sarcomere length and tenderness (Bouton, *et al,* 1973; Sorheim & Hildrum, 2002).

For the AD, VL, RF, and PM muscles they found low but significant ($P<0.05$) negative correlations of -0.26 to -0.36 between sarcomere lengths and shear force values. Very low ($P>0.05$) negative correlations were found for the *Semimembranosus* (SM), *Biceps femoris* (BF) and *Gluteus medius* (GM) muscles, whilst a positive correlation, indicating longer sarcomeres coinciding with higher shear force values, were found for the *Semitendinosus* muscle. This was in contrast to the studies by Hostetler *et al,* (1970, 1972, and 1973) although they noted a positive correlation for the *M. biceps femoris*. They postulated that the reason for lower tenderness levels with an elongated sarcomere length is attributed to the amount of connective tissue present in the muscle (Herring *et al,* 1967). In addition, Marsh and Carse (1974) found a "peak" of toughness when muscles were held at a 25-30% extended state during rigor onset (As cited by Shanks *et al,* 2002).

An explanation for increased shear force values coinciding with increased sarcomere length could be that excised muscles that were allowed to contract had less muscle fibres and a smaller percentage endomysial and perimysial material per unit area than muscles at rest or stretched lengths. This meant

that contracted muscle, although known to be tougher, still had less endomysial and perimysial connective tissue per cross section than a stretched muscle. However, it could be explained that when shearing through a stretched muscle, more fibres, endomysium and perimysium must be cut per unit area than through a similar cut through a contracted muscle (Herring *et al,* 1967). When Torrescano *et al,* (2003) assessed the correlation between collagen content and 14 bovine muscles, a very weak correlation was found between sarcomere length and WBSF values over all 14 muscles.

In 1994 Wheeler and Koohmaraie found a reduction in sarcomere length for sheep *Longissimus* muscles during the rigor phase coinciding with increased toughness. From this the idea came forward that toughening during rigor is a result of sarcomere shortening during 0 – 24 hours post mortem.

They tested this theory by showing that, before the aging process begins, there was a decrease in muscle tenderness. A maximum toughness was recorded between 9 and 24 hours post mortem and a large amount of variation in tenderness existed after the first day of post mortem storage (Koohmaraie, 1995, 1996; Savell *et al,* 2005).

Koohmaraie, (1996) indicated that by preventing the shortening of sarcomeres during the rigor period no subsequent rigor toughening occurred. The outcome of this study showed that sarcomere shortening could be the cause of meat toughening during the first 24 hours post mortem. This however did not prove that shortening of the muscle fibres was the reason for toughening but rather that post mortem meat toughening was lessened in the absence of shortening.

Sarcomere length is therefore mostly a measure on the effectiveness of the restraining or stretching of the muscle, since long sarcomeres do not always indicate tender meat. (Sorheim & Hildrum, 2002)

A linear relationship was found between the changes in sarcomere length and the fibre diameter (Herring *et al,* 1965b). Hiner *et al,* (1953) demonstrated that fibre diameter had a curvilinear effect on shear force for a number of muscles. Herring *et al,* (1967) also made the connection between decreased sarcomere lengths and an increased fibre diameter. A distinct increase in fibre diameter was documented with every increment reduction of sarcomere length, especially in the lengths shorter than 2.0μm. Between lengths of 2.0 and 3.25μm however, a scatter diagram depicting the effect of sarcomere length on fibre diameter through regression equations illustrated a relatively flat slope which indicated that only a small increase in tenderness occurs when sarcomeres are stretched beyond 2.0μm (As cited by Herring *et al,* 1967).

Marsh and Leet (1966) suggested that the subsequent toughness of contracted muscle was more a result of the actomyosin formation within the fibres itself than the contribution of “background” toughness due to the impact of connective tissue (Herring *et al,* 1967). In earlier studies, Goll *et al,* (1995)

(as cited by Koohmaraie, 1996) claimed that actin/myosin interactions changed during the first 24 hours post mortem from a weak binding state, to a strong binding state, causing subsequent toughening. They hypothesized that the shortening of sarcomeres only intensified these bonds and were not directly responsible for toughening. Since Koohmaraie (1996b) found that no toughening occurred when sarcomeres were prevented from shortening, it was thought that these changes in actin/myosin interaction were perhaps only responsible for the toughening in the early post mortem stages where no sarcomere shortening took place (Koohmaraie, 1996).

The work of Locker (1960), Marsh and Leet (1966), Herring *et al,* (1965a, b; 1967a, b), Davey *et al,* (1967) and Koohmaraie (1996b) showed that the state of contraction of the muscle fibres greatly influenced the tenderness and that the role of connective tissue was demoted to that of background toughness. It is now established that both the fibres and its associated connective tissues contributes to toughness. Thus the influence of aging upon both these structural components must be taken into account when investigating the effects of aging (Bouton and Harris, 1972).

From aforementioned data it appears that Hostetler *et al,* (1972) was correct by claiming that there was much more to meat tenderness than just sarcomere length (as cited by Shanks *et al,* 2002).

2.3 Post mortem aging

The process of aging refers to the increased palatability gained when meat is held post mortem under optimum conditions for a period of time along with specific degradation of the structural proteins (Thompson, 2002; Hwang *et al,* 2003).

This increased palatability or the degree of increased tenderisation is influenced by various factors such as species, physiological maturity, diet, sex, the anatomical location of the cut, the degree of muscle shortening during rigor and the aging temperature (Jeremiah & Martin 1977; Fapohunda & Okubanjo 1987; Huff & Parrish 1993; O'Connor *et al,* 1997; Sanudo *et al,* 2004; Braghieri *et al,* 2005; Derbyshire *et al,* 2007)

Believing that connective tissue was the structural component responsible for the tenderness level of the meat meant that it was also thought that post mortem aging of the meat, just above freezing point, alters this connective tissue to produce more tender meat during the aging process (Lehmann & Rumpf, 1907; Mitchell *et al,* 1972; Mackintosh *et al,* 1936; as cited by Bouton and Harris, 1972). Goll *et al,* (1970) stated that aging caused changes in the strength and number of cross-bridges between the

collagen molecules and that these cross linkages weakened and ruptured during the aging process that subsequently increased the collagen solubility when the meat was cooked at temperatures between 50 - 80°C.

Bouton and Harris (1972) ruled out the possibility that changes in connective tissue during aging adds to its increased tenderness level when the process of aging did not significantly affect the adhesion values of the meat and when the changes in connective tissue were simply too small to be singled out by any mechanical measurements. Therefore, the significance of connective tissue toughness only applies when the muscle fibres themselves have already decreased in toughness during aging. This is why muscles with high natural connective tissue toughness can remain tough even after a period of aging.

As a result, Hanson *et al,* (1942) and Ramsbottom and Strandine (1949) stated that the muscle fibres as well as the connective tissue fibres were affected by aging when Hoagland *et al,* (1917) as cited by Herring *et al,* (1967) focussed more on enzymatic degradation. Stromer and Goll (1967a, b) and Stromer *et al,* (1967) showed that ageing affected the myofibrils by creating structural changes to the Z-line area and the I-bands causing the myofibrils to weaken. In addition Aberle and Merkel (1966) as cited by Herring *et al,* 1967 found that increased fibrillar protein solubility achieved by aging had a positive correlation with reduced shear force values. Davey *et al,* (1967) and Herring *et al,* (1967b) however felt that the state of muscle fibre contraction played an important role in the amount of tenderisation post mortem aging imparts on the meat.

Additionally, Partman (1963) thought that changes in the tenderness level were caused as a result of the dissociation of the actomysin complex. Besides the various reasons postulated, Taylor *et al,* (1995) and Savell *et al,* (2005) as cited by Thompson *et al,* (2006) suggested that a change in the strength of the actin-myosin binding sites during the first 24h post mortem were responsible for the fibre shortening during this period.

Sarcomere length and ageing

Smulders *et al,* (1990) analysed aged (14 days) and un-aged (24 hours post mortem) beef meat for the correlation between sarcomere length and tenderness where high correlations (r = -0.50) were found for all the un-aged meat. Carcasses were divided into two groups according to the pH measurements taken 3 hours post mortem. In the group where the pH was above 6.3, a strong correlation was demonstrated between the sarcomere length and the shear force for both aged (r = -0.80) and un-aged (r = -0.84) meat. The second group with a 3 hour post mortem pH value below 6.3, showed no relationship between these two factors.

From this study these researchers postulated that a high correlation between sarcomere length and tenderness only occurred in slow glycolysing muscles, whilst in fast glycolysing muscles the tenderness was completely independent to the degree of sarcomere shortening. This independent response of tenderness to sarcomere length in fast glycolysing muscles was explained by an increased aging rate from these carcasses. It was therefore understood that the relationship between sarcomere length and tenderness was strongly influenced by the degree of post mortem tenderisation. After a substantial time of aging, a very low relationship between sarcomere length and tenderness can therefore be expected as well as the opposite, being a high relationship coinciding with low post mortem tenderisation (Table 1) (Koohmaraie, 1996).

This indicates that the rigor-induced sarcomere shortening is mainly responsible for meat toughening occurring during the first 24 hours post mortem. The amount of correlation between sarcomere length and tenderness 24 hours post mortem is therefore dependent on the extent of tenderisation occurring during the shortening phase of rigor. Thus the shear force of a muscle at any given time is a direct result of the relationship between the two conflicting agents, sarcomere shortening and tenderising. Therefore, it is either necessary to minimise the toughening phase or to improve or accelerate the tenderisation phase for gaining the maximum level of tenderness (Koohmaraie, 1996).

Table 2.1 Effect of the length of post-mortem storage on the correlation between sarcomere length (SL) and shear force measurements for lamb meat

Time Post mortem	n	SL mean µm	SL range µm	Shear force mean, kg	Shear force range, kg	r
1d	30	1.70	1.43-1.89	7.85	3.88-12.90	-0.52
3d	19	1.72	1.59-1.86	4.61	3.22- 6.36	- 0.31
14d	20	1.83	1.52-2.26	2.79	1.72- 4.60	0.12

Based on data from Wheeler and Koohmaraie, 1994 as cited by Koohmaraie, 1996

Other authors postulated that the process of aging and tenderisation was the result of autolysis causing the coagulation of the muscle proteins and the softening and sealing of the collagen fibres which, through the action of lactic acid, was transformed into softer and more digestible gelatine (Gracey and Collins 1992 and Geesink *et al,* 1995; as cited by Derbyshire *et al,* 2007).

Despite these various suggestions for post mortem tenderisation, consensus between numerous authors has been reached that post mortem proteolysis was the result of the degradation of proteins responsible for sustaining the structural integrity of the myofibrils causing a weakening of these myofibrils and therefore subsequent tenderisation Penny, 1980; Davey, 1983; Goll *et al,* 1983; Greaser, 1986; Koohmaraie, 1988; Ouali, 1990; Goll, 1991; Koohmaraie 1992a; Ouali, 1992; Koohmaraie 1994; Goll *et al,* 1995; Koohmaraie *et al,* 1995; Taylor *et al,* 1995; as cited by Koohmaraie, 1996

For proteases to be involved with post mortem tenderisation, it needs to meet certain criteria. A protease must be a part of the skeletal muscle cell. It must have the ability to reproduce the same effects of post mortem tenderisation on myofibrils in-situ as well as in-vitro under optimal conditions. Lastly, a protease must have access to the myofibrils within the tissues. All of these factors are essential for any protease to be considered as a part of the tenderisation process (Koohmaraie, 1996).

Calpains

Koohmaraie, (1988, 1992a, b and 1994) claimed that calpains, specifically µ-calpain, consist of all three of these requirements and was therefore the most important factor in post mortem tenderisation. A number of studies agreed that µ-calpains were the main factor responsible for tenderisation post mortem (Penny, 1980;Goll, 1991; Goll *et al,* 1983, 1995; Koohmaraie, 1988, 1992a, 1994, 1995; Koohmaraie *et al,* 1995; Ouali, 1990, 1992; Taylor *et al,* 1995; Koohmaraie, 1996) and even though Hopkins and Thompson (2002) did not fully understand the workings behind the effects of aging, it was concluded that tenderisation was mainly a function of the calpain system (as cited by Thompson, 2002).

Calpains, which are Ca^{2+} dependent proteases, were believed to be mainly responsible for the breakdown of certain structural proteins at the Z-line (Gault, 1992 as cited by Thompson *et al,* 2006). Although calpains degrade the major structural proteins of the muscle (Taylor *et al,* 1995) they also digest themselves and their inhibitor, calpastatin. It is because of this inactivation of calpains, that the extent of tenderisation is determined by the net proteolytic activity of both these enzymes (Tornberg, 1996; Thompson *et al,* 2006).

Enzymatic degradation, due to the work of calpains and lysosomal proteases is influenced by the temperature it is stored at, the pH, the muscle fibre type, the amount and type of cross linkages in the connective tissue and the animal breed or species (Smulders *et al,* 1992; Savell *et al,* 2005).

Of the protease enzymes, the calpains are very sensitive to the pH and temperature of the meat during the conditioning stage (Dransfield, 1994b). A combination of low pH and high temperatures pre-rigor has been shown to increase the level of autolysis and degradation of the μ-calpains and therefore inhibits the aging potential (Dransfield, 1994a; Ducastaing, *et al*, 1985; Geesink, *et al*, 1994; Hwang & Thompson, 2001; as cited by Thompson *et al,* 2006).

During slow chilling regimes that include a rapid pH decline, the activity of μ-calpains and calpastatin decreased substantially, however with fast chilling, their activity stayed unaffected by the rate of pH decline (Hwang & Thompson, 2001; Thompson *et al,* 2006) A rapid glycolytic rate increases the activity of μ-calpains and calpastatin by early activation of the calpain system. This early activation was highly correlated with an increased self-destruction of the enzymes, which was aggravated by a rapid pH decline at high temperatures (Hwang *et al,* 2003). For these reasons temperatures between 10-25°C were suggested to be the most favourable for calpain activity (Dransfield, 1994a).

Certain properties of the μ-calpain system were thought to contradict its involvement with post mortem tenderisation. The first contradiction was that μ-calpains are very rapidly inactivated which makes them unlikely to be responsible for degradation after 24-48 h post mortem. Challenging this statement, Koohmaraie, (1992) used radio labelled casein, a very sensitive quantification method, and found sufficient amount of μ-calpains (5-10%) remaining after 14 days of aging at 4ºC. This was due to the fact that the autolysis and inactivation of μ-calpain is an intermolecular process whilst the same process for m-calpains is intracellular (Cottin *et al,* 1986; Inomata *et al,* 1988; Edmunds *et al,* 1991; Nishimura & Goll, 1991; Koohmaraie, 1992). Therefore no activated m-calpain would be found after an extensive period of aging. M-calpain will undergo rapid autolysis with the slightest amount of calcium available, thus making it clear that after post mortem injection with calcium chloride to improve tenderisation, it is the μ-calpains

that are triggered as most of the m-calpains have already been inactivated. Thus it can only be μ-calpains responsible for long post mortem period tenderisation and not m-calpain (Koohmaraie, 1996).

The second conflicting property about μ-calpains was the existence of almost double the amount of calpastatin in the muscle, inhibiting the work of μ-calpains and making it almost impossible for this enzyme being responsible for the degradation post mortem. These ratios were normally assessed by using m-calpains to quantify the calpastatin activity. When Koohmaraie (1996) used μ-calpains as a measurement to quantify the calpastatin activity rather than m-calpains as normal, the ratios were almost half i.e. 2:1 for beef, 1.25:1 for lamb and 0.75:1 for pork in accordance with 4:1, 2.5:1, 1.5:1 in beef, lamb and pork, respectively. The reason for this was that it takes almost double the amount of calpastatin to inactivate μ-calpains than that required to inactivated m-calpains (Koohmaraie, 1996).

From this it can be assumed that having a higher calpain to a lower calpastatin ratio ensures ideal opportunities for producing tender meat, however there were more variations in tenderness due to processing procedures than the levels of calpains, which led to the conclusion that maybe too much emphasis was placed on calpain in terms of its quantitative effects. Much lower calpain levels exist during high rigor temperatures (35ºC) than at lower rigor temperatures (15ºC) and become even lower and more variant during post mortem aging (Simmons *et al,* 1996). McDonah (1998) showed that the calpain-calpastatin activity still occurs during cold shortening; however the meat does not arrive at an acceptable tenderness level, which signifies the ability of the structural conditions to overrule the effect of the calpain system. This questioned the significance and relevance of the pre-slaughter calpain and calpastatin levels for the prediction of meat quality (Devine, 2001). It was noted that only 30% of the post-mortem tenderisation can be explained by the calpain system and that any structural conditions can undo any processing advantage it has for tenderness (McDonagh, 1998). Considering this, the calpain levels might indicate the potential quality of the meat, however without addressing all the processing variables first, it is rendered futile (Devine, 2001).

Effect of number of days aging

Eilers *et al,* (1995) characterised the effect that the number of days of aging, has on different meat cuts. For the shear force measurements of the LM muscle a significant cubic effect of aging was found, which showed a reduction in the shear force values between days 6 and 12 and between days 18 and 24 post mortem. No changes were observed between the 12^{th} and the 18^{th} day. The ratings on panel tenderness for the LM steaks increased ($P<0.01$) quadratically as the aging period increased with a large improvement between days 6 and 12 post mortem and a slower tenderisation rate from day 12 onwards. For GM steaks both shear force and panel tenderness had linear responses to increased time of aging

post mortem. The tenderness increased (P<0.01) at a constant rate up to 24 days of aging. Again a quadratic improvement was observed for SM steaks with a sharp reduction in shear force between days 6 to12 and a more steady decline between 12 to 24 days post mortem. The Panel tenderness ratings for the SM steaks increased steadily and linearly over the whole range of aging time from days 6 on through until day 24.

Therefore, to ensure that meat reaches an acceptable level of tenderness some suggestions were made on the aging time for certain cuts. It was recommended that strip loins should be aged for at least 12 days to gain an “acceptable” shear force value (<3.9kg) or for a longer period of 24 days for a “superior” (<3.2kg) tenderness level (Eilers *et al,* 1995). Eilers *et al,* (1995) reported 70% of LM steaks measuring a shear force of beneath 3.2kg after being aged for 24 days (Eilers *et al,* 1996). After 6 days of aging shear force values were ≥3.9kg for 59.4% of the GM steaks. After 18 days of aging, 34.4% of GM steaks had a shear force value of 3.9kg and higher, 16% had shear force values equal and lower than 3.9kg whereas 46% had lower than 3.2kg after 24 days of aging. Fewer than 20% of the SM steaks had a shear force value below 3.2kg at 24 days however a decrease in the within-class variation was observed for SM steaks as the aging period increased from 6 to 24 days. With this study they came to the conclusion that subsequent toughness of top rounds and top sirloins will be significantly reduced, by aging them for at least 12 and 24 days, respectively.

Chapter 3

Factors affecting beef tenderness

It was believed that the increased demands for efficient slaughtering and chilling of carcasses with regards to increased hygiene and decreased weight loss through reduced drip loss, was responsible for the manifestation of "unacceptable" tenderness levels – the meat was too tough (Sorheim and Hildrum, 2002).

A Total Quality Management system (TQM) was recommended by Tatum, *et al* (1999) to control and reduce the variance in the acceptability of beef tenderness by applying the best genetic programme, ante mortem management system, early post mortem processing procedures and post mortem aging. It was guaranteed that by using this TQM system, the occurrence of tough meat, evaluated by shear force, would be reduced (As cited by Thompson, 2002).

There are several points in a beef production system where management decisions can either improve or reduce the subsequent quality of the product. These points that control whether the meat obtains its acceptable level of tenderness are called "critical control points" (CCP's) (Thompson, 2002) and can also be used to predict the subsequent tenderness of the meat being produced (Tatum *et al,* 1999). These CCP's can incorporate areas of production from selection of your breed up to how the food is cooked. The first four CCP's are of most importance and include; genetic inputs, pre slaughter management, early post mortem management and post mortem aging. These will be discussed further in more detail.

3.1 CCP 1 – Genetic inputs

A genetic basis exists for the differences in tenderness and intramuscular fat content for most animals (Shackelford *et al,* 1994; Wulf *et al,* 1996b; O'Conner *et al,* 1997). Differences in tenderness between specifically *Bos taurus* and *Bos indicus* have been repeatedly documented (Crause *et al,* 1989; Sherbeck *et al,* 1995; Wheeler *et al,* 1996) however it was also found that there were more differences in tenderness within breeds than between breeds (Wheeler *et al,* 1996; Wulf *et al,* 1996b; O'Connor *et al,* 1997). Therefore it was claimed to be more effective to breed specifically for tenderness and marbling which are heritable traits within breeds (Koch *et al,* 1982; Green *et al,* 2000; Tatum *et al,* 1999).

Most studies documented the amount of *B*. *indicus* strain within a crossbreed. It has been reported numerous times that a high *B*. *indicus* content in cattle would be more inclined to produce lower

marbling scores and less tender, more variable striploin steaks than *B*. *taurus* breeds (Crouse, Cundiff, Koch, Koohmaraie, & Seideman, 1989; Hearnshaw *et al,* 1998; Wheeler, Cundiff, & Koch., 1994; Thompson, 2002). Variation between studies exists since Morgan *et al,* (1991) showed that 25% of the breed must be of *B*. *indicus* content before the consumer can detect a decline in the palatability score whereas Sherbeck, Tatum, Field, Morgan, & Smith (1995) claimed it to be 50% and Rymill (1997), 75% (as cited from Thompson, 2002).

Shackleford, Wheeler, and Koohmaraie (1995) did a study on the *B*. *indicus* content and cut interaction on palatability and its effect was only significant for the *M. triceps brachii, longissimus dorsi, supraspinatus, biceps femoris,* and the *quadriceps femoris*. However in the study from Thompson, Polkinghorne, Hearnshaw , & Ferguson (1999), a more dominant effect on the loin muscles especially the *M. psoas major* were found where a regression coefficient was made for the palatability score of different muscles as a function of the percentage *B*. *indicus* content after a 14d aging period. This established that there was a definite interaction between breed type and muscle and that the palatability decreased with at least ten points with an increase in the amount of *B*. *indicus* content from 0 to 100% (Thompson, 2002).

3.2 CCP 2 – pre-slaughter production management

3.2.1 Age at slaughter

Studies from Jacobsen and Fenton (1956) and Goll *et al,* (1963) showed that with increased animal age, shear force values increased and panel tenderness levels decreased. According to Tuma *et al,* (1963) as cited by Herring *et al,* (1967) the higher amount of perimysium in older animals had an influence on the higher shear force and lower panel tenderness levels. This was due to the structural changes in the collagen as the animal matures (Goll *et al,* 1963). Hill (1966) described it as an increased number, or strength of the cross linkages of the intramuscular collagen which means that the collagen becomes less soluble with age. Chemical studies have not shown a significant difference in connective tissue content between young and older animals (Goll *et al,* 1963; Hill, 1966; as cited by Bouton and Harris, 1972).

Herring *et al,* (1967) found that age had a highly significant ($P<0.01$) effect on shear force and panel tenderness. Jacobsen and Fenton (1956) and Goll *et al,* (1963) also reported an increase in shear force values and decreased organoleptic scores for tenderness and juiciness with increased animal age (As cited by Herring *et al,* 1967).

3.2.2 Endocrine status of the animal

Probably the most important factor influencing the deposition of marbling and beef tenderness in the pre-slaughter management stage is those practices that alter the endocrine status of the animal (Tatum *et al,* 1999). Dikeman (1987) as cited by Thompson, (2002) postulated that 10-15% of the variance in palatability is accounted for by marbling.

Testosterone

Castration of male cattle is probably the most popular way of endocrine modification (Unruh, 1986). Elevated serum testosterone levels and therefore sexual development at ages 8 to 14 months caused a significant increase in the intramuscular collagen content, which means less tender meat in intact bulls than in steers (Cross *et al,* 1984; Boccard *et al,* 1979; as cited by Tatum *et al,* 1999).

Calpastatin

Besides the testosterone level, a higher calpastatin activity exists in bulls than in steers, inhibiting the work of the calpain system, causing slower tenderisation during the aging period which therefore could produce tougher meat than that from steers (Morgan *et al,* 1993; Tatum *et al,* 1999).

Exogenous Hormonal growth promoters **(HGP)**

Implants are used to increase the growth rate and the efficiency of feed utilisation of the animal. It has been proven that when these implants, especially those containing very powerful anabolic agents, are used repeatedly or too close to the slaughter date, it could have a negative effect on the tenderness level of the meat and reduce the deposition of intramuscular fat (NLSMB, 1995; Morgan, 1997; Roeber *et al,* 1999; Tatum *et al,* 1999). In addition, these implants are responsible for decreased marbling scores and an increased occurrence of dark cutters (Duckett *et al*, 1997; Hunter *et al*, 2001). The effect of HGPs on tenderness varies considerably since Huck *et al*, (1991) and Hunter *et al*, (2001) showed no effect on objective and sensory measurements, respectively. However, Roeber *et al,* (2000) found negative effects for both these measurements.

Pre-slaughter factors such as the number of days the animal is fed a high-energy diet (Tatum *et al,*1980; Dolezal *et al,* 1982; Van Koevering *et al,* 1995), the health status of the animal during its growing and finishing periods (Gardner *et al,* 1999), age at castration (Martinez-Peraza *et al,* 1999), intramuscular injection of animal health products (George *et al,* 1995), temperament or ante mortem stress (Voisinet *et*

al, 1997), age (Wulf *et al,*1996a) and relative fatness of the animal at slaughter (Dikeman, 1996) (Tatum *et al,* 1999) have repeatedly been proven to have a great influence on the tenderness of the meat.

For this reason, the NCBA Beef Palatability Task Force in the U.S implemented quality management practises to eliminate the effect of some pre-slaughter stresses. These include the elimination of the excessive use of anabolic implants; discontinuing the use of biological types of breeds prone to producing tough meat; the exclusion of intramuscular injections; the slaughtering of cattle before reaching the age of 30 months, the castration of bull calves earlier than seven months of age and putting an end to feeding programs of less than a 100 days for large biological type cattle (Tatum *et al,* 1999).

3.2.3 Pre slaughter stresses

Variances between the amount of stress experienced between different animals are a result of the numerous different types of stressors which can broadly be classified as being physiological (restraint and handling) or physical (hunger, thirst, fatigue, injury or thermal extremes) (Grandin, 1997).

The problem with managing stress is that it is difficult to determine. The extreme effects from stress can only be determined through observing the changes in the animals' gross behaviour patterns or to establish whether the animal experienced any stress before any of the effects that the stress could create is observed. (Devine, 2001) Howard and Lawrie (1956) showed that exercise alone does not raise the end pH level, but that elevated pH levels were found when exercise was combined with other stressors such as transport and animal mixing (Wythes & Shorthose 1984). (Devine, 2001) Stress and its subsequent elevated pH level not only affect the tenderness of meat, but higher ultimate pH levels also affect the denaturation of myoglobin during cooking. This generates a problem when creating a repeatable degree of "doneness" or "appropriate colour" in the food service industry (Cox *et al,* 1994).

Due to its invisibility, stress is normally ignored and its effects only revealed after it has occurred, creating a lower pre-slaughter muscle glycogen level (Devine, 2001). Therefore pre-slaughter management's one obligation is to reduce stress and minimise the depletion of glycogen reserves prior to slaughter (Thompson, 2002). These management practises should be implemented on the farm, during transport and in lairage.

Ferguson *et al,* (2001) concluded that the emotional state of the animal has a much greater effect on the depletion of glycogen than a low physical activity such as transport, especially if the distances travelled are less than 400km. The effect of transport varies with the type of animal, the nutritional status of the animal and the conditions during transport (Tarrant, 1990; Thompson, 2002).

Cattle that are well fed just before transportation should have a muscle glycogen concentration of between 60 and 120µmol/g (Pethick *et al,* 1999) to achieve the optimum end pH level of 5.7 (Tarrant, 1989; as cited by Thompson, 2002).

Mixing of cattle has been proved to bring upon a number of dark cutters (DFD) especially in bulls, due to the high mobilization of glycogen (Grandin, 1993) brought about by the bulls sorting out their rank in the new environment.

3.3 CCP3 - Early post mortem management

3.3.1 Onset of rigor

Before slaughter, ATP and creatine phosphate within the muscle supply energy for metabolism. Post mortem, the muscle continues to metabolise, however with the termination of blood circulation at death, anaerobic metabolism of glycogen falls into place to replace the ATP reserves. Without glycolysis no ATP is produced for the still ongoing muscle metabolism and ATP reserves will be depleted. This ATP depletion is called the onset of rigor mortis (Bendall 1969 as cited by Thompson *et al,* 2006).

With the cessation of blood circulation, waste products remain in the tissues where the build-up of lactate and its related hydrogen ions lower the pH of the muscle from neutral to slightly acidic (Marsh, 1993; Thompson *et al,* 2006). When glycogen reserves are depleted and the pH becomes too acidic for the enzymes to function, glycolysis seizes to function. (Lawrie, 1992; Thompson *et al,* 2006) The lactic acid build up resulting from anaerobic glycolysis, lowers the pH to a range between 5.7 – 5.8 where rigor mortis begins (Hannula & Puolanne, 2004). Permanent cross bridges called the actomyosin complex form between the actin and myosin filaments causing the muscle fibres to stiffen. The ATP left in the muscle, binds with Mg^{2+} and breaks the actomyosin complex, which in turn makes the muscle relax again. During the depletion of creatine phosphate the phosphorylation of ADP to ATP is inhibited. The lower ATP concentrations during the onset of rigor therefore reduce the breakage of all the actomyosin cross bridges, causing the muscles to stay inextensible (Aberle *et al,* 2001). This onset of rigor occurs per individual muscle fibre as its own ATP reserves becomes depleted and not as a whole muscle (Hwang *et al*, 2003). For this reason the overall muscle stiffness will steadily increase as each muscle fibre experiences its own rigor contraction. (Honikel *et al*, 1983; Jeacocke, 1984; Thompson *et al,* 2006).

3.3.2 Rigor shortening

As previously stated, rigor mortis refers to the state of stiffness in every single fibre as it goes into full rigor resulting from irreversible cross bridges that form between the contractile components actin and myosin when all the fibres were depleted of their supply of energy (ATP) which could cause toughening of the meat. (Bendall, 1969; Hwang *et al,* 2003)

Early post-mortem, two factors work together to influence the tenderness level of beef carcasses namely, the "rate of post mortem glycolysis" and the "rate of cooling" (Lee, 1986; Marsh *et al,* 1987; Geesink *et al,* 1995; Tatum *et al*, 1999). Both of these variables can be manipulated by post mortem management, (process control). For example, air temperature and air velocity can be altered to manipulate the chill rate of the carcass and the rate of glycolysis can be increased by using electrical stimulation (Marsh *et al,* 1988; Mallikarjunan & Mittal, 1995; Tatum *et al,* 1999).

3.3.3 Glycolytic rate

Muscle glycogen concentration

The glycolytic rate, which is determined by the rate of ATP hydrolysis via various muscle ATPase systems, has a direct correlation to pH decline. The most important factor in the glycolytic rate is the amount of glycogen (glucose 6-phosphate) available within the muscle (Thompson *et al,* 2006). Thus the higher the glycogen concentration in the muscle, the higher the rate of pH decline which means that the muscle will reach rigor mortis (pH 6.0) at a higher temperature. (Daly *et al*, 2002) as cited by Thompson *et al,* 2006) In slow glycolysing beef carcasses, tenderness was improved by lowering the chill rate or by using "delay chilling regimes". A negative effect of this slower chill rate is an increased probability of high micro organism counts (Dutson, 1977; Lochner *et al,* 1980; Lee & Ashmore, 1985; Tatum *et al,* 1999).

Cattle that are well fed just before transportation should have a muscle glycogen concentration of between 60 and 120 µmol/g (Pethick *et al,* 1999). Tarrant (1989) stated that for an animal to achieve an ultimate pH of 5.5 post slaughter, it must have at least 57µmol/g of glycogen in the pre-slaughter muscle so that an adequate amount of lactic acid is formed to sufficiently lower the ultimate pH. A high ultimate pH (e.g. 6.0) will result in meat that is dark in colour, less juicy, has a reduced shelf life (Shorthose, 1989) and is also tougher (DFD) (Purchas & Aungsupacorn, 1993). The Meat standards Australia (MSA) suggested a maximum ultimate pH of 5.7 to ensure tender meat with an attractive exterior and a substantial shelf life (Thompson, 2002).

It has been documented that feedlot cattle have a higher buffer of glycogen in their muscles and therefore lose less glycogen than pasture fed cattle during activities such as loading, transport and lairage. A number of techniques can be implemented to minimize the amount of glycogen lost from stressors or to increase the initial glycogen concentration of the muscle (Pethick *et al,* 1999). One of these techniques includes a short-term grain fed period prior to slaughter used together with a suitable rumen modifier as to control acidosis (Gardner, 2000; Thompson, 2002).

The MSA audited abattoirs and found that for grain fed carcasses, less electrical stimulation is needed to reach the same glycolytic rate than pasture fed and the same principles are applicable for larger compared to smaller carcasses (Thompson, 2002).

Carcass weight

Carcass weight has a primary effect on glycolytic rate through the effect that temperature has on glycolysis. A linear effect exists between carcass weight and the rate of temperature fall per hour. The rate of temperature fall will decrease with 0.05°C per hour for every 20kg increase in carcass weight (Daly, 2005). This increase in muscle temperature causes a faster rate of pH decline so that for every 20kg increase in carcass weight the temperature at pH 6.0 will increase with 1°C. This means much higher temperatures at pH 6.0 which could mean a higher incidence of heat rigor shortening (Thompson *et al,* 2006).

Genotype

The effect that genotype has on the glycolytic rate is mainly attributed to the difference in muscle fibre types. Studies have shown that the bigger the proportion of type IIB fibres in a muscle, the faster the rate of pH decline. Animals that are selected for a fast growth rate and a high feed efficiency showed a higher proportion of fast glycolytic to slow glycolytic fibres than in those animals with slower growth rates and lower feed efficiencies (Thompson *et al,* 2006).

Variation

Beside these factors causing variations between groups in glycolytic activity, there are still numerous amounts of variation within groups. Within lot carcasses the average variation in temperature at pH 6.0 was 4.2°C. The variation ranged between 1.3 and 8.3° which indicates that although the mean glycolytic rate coincides with the optimal pH/temperature window there might be many carcasses that could run the

risk of heat or cold shortening. This high variance in temperature of the carcasses at pH 6.0 makes it difficult to predict or optimise the subsequent eating quality of the carcass group or lot (Thompson, 2002).

3.3.4 Post mortem environmental effects on muscle structure

In the conversion of muscle to meat the first 24 hours post-slaughter is probably the most important and influential in the attaining of positive meat quality traits such as tenderness and colour (Savell *et al,* 2005).

3.3.4.1 pH/temperature window

Consequently, Locker and Hagyard (1963) started the concept of the pH/temperature window (Figure 3.1) by showing myofibrillar shortening occurring whilst the pre-rigor muscles were subjected to high and low temperatures (As cited by Thompson, 2002).

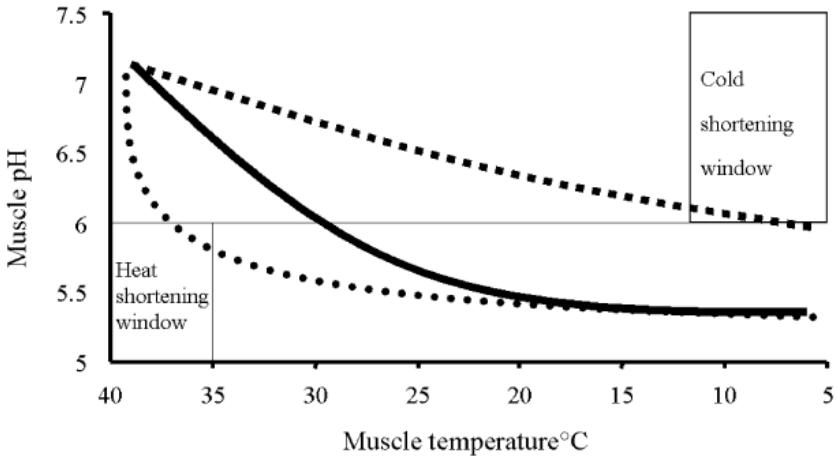


Figure 3.1. The pH/temperature window used by MSA to optimise the decline in pH relative to the temperature of the muscle. The solid line represents an optimal rate of decline, the dashed line a cold shortening and the dotted line a heat shortening scenario (adapted from Thompson, 2002)

The degree of myofibrillar contraction is directly dependent on two key factors namely the rate of pH decline and its correlation to the temperature at the onset of rigor mortis (O' Halloran *et al,* 1997; Hannula & Puolanne; 2004) Normal pH decline ranges from 7.0 upon slaughter to 5.3 – 5.8 at rigor. The rate of pH decline differs between species where for example pork takes about 6-12 hours, and beef range between 18-40 hours to reach its end pH (Smulders *et al,* 1992).

pH

Bouton *et al* (1971) and Purchas (1990) noted that both high and low ultimate pH results in tender meat whereas an intermediate pH level (pH 5.9 – 6.0) was responsible for tougher meat. In fact there was a dramatic climb in tenderness in the pH range 5.5 – 5.8, which is the normal ultimate pH range found in commercial beef carcasses and accounts for up to 50% decrease in shear force values in un-aged meat. (Devine, 2001) Other studies have specifically shown that a low pH level (below 6.2 – 6.3) at 3 hours post mortem is associated with a decreased variation in tenderness of beef loin steaks (Smulders *et al,* 1990; Jones & Tatum, 1994). Eilers *et al* (1995) and Jones and Tatum (1994) again showed that carcasses with lower early post mortem muscle pH values will produce more tender meat (As cited by Eilers *et al,* 1996). The rate of pH decline thus has an inverse effect on the tenderness of the meat (Howard & Lawrie 1956).

Temperature

The extent of post mortem shortening and toughening of the meat is also greatly dependent on the temperature at rigor. Locker and Hagyard (1963) as cited by Sorheim *et al* (2001) tested this theory by measuring the shortening of beef *M. sternomandibularis* over a range of constant rigor temperatures between 2°C and 37°C. The results showed that at temperatures in the range between 14°C to 19°C the minimum shortening occurred. Lower and higher temperatures resulted in shortening of the muscle fibres, higher temperatures giving less contraction than colder temperatures.

An optimal temperature of 15°C was therefore suggested by Locker and Hagyard (1963) for when the muscles enter rigor mortis. It has also been found that the lowest shear force values were obtained at this temperature (Tornberg, 1996). Above this temperature the fibres start to contract at rigor (rigor shortening) and at temperatures below this optimum the muscle fibre contraction occurs before rigor and continues throughout the rigor process creating a much stronger reaction called cold shortening (Hwang *et al,* 2003) (As cited by Thompson *et al,* 2006).

3.3.4.2 Temperature induced shortening

Cold shortening

The phenomenon has been studied since the 1960s when Locker and Hagyard (1963) reported that cold shortening occurred when the muscle temperature rapidly decreases below 14 to19°C before rigor mortis has begun its first phase. In addition, Bendall (1973) and Pearson and Young (1989) found that muscles exposed to temperatures less than 10°C before the onset of rigor were more inclined to undergo cold

shortening than those muscles chilled at 16°C at a pH less than 6.2 and therefore recommended that carcasses should not be cooled below 12°C internally (As cited by Savell *et al,* 2005).

Temperatures below 12°C before the onset of rigor causes a pre-rigor shortening due to increased sarcoplasm calcium levels excreted from the sarcoplasmic reticulum as the cold temperature inhibits the ATP dependent calcium pumps to re-absorb the free calcium and in turn activates the actomyosin ATPase complex responsible for contraction and the meat's subsequent toughness. (Pearson *et al,* 1973; Kanda *et al,* 1977; Bendall, 1978; Honikel & Hamm, 1978; Hwang *et al,* 2003 and Savell *et al,* 2005 as cited by Thompson *et al,* 2006). The binding between actin and myosin is then stimulated by this increased cellular calcium levels, which, as previously stated, activates the Ca^{2+} -dependent myosin ATPase, which energizes muscle contraction by hydrolysing ATP. (Thompson *et al,* 2006) According to Aberle *et al* (2001) the sarcoplasmic reticulum is least efficient at temperatures between 1°C and 2°C. Savell *et al* (2005) stated that the degree of cold shortening is basically inversely correlated to the extent of rigor the muscle reaches at the onset of the chilling regime.

Cold shortening has less effect on white muscle fibres than red fibres due to the fact that white muscle fibres have a higher glycogen concentration and therefore have a more severe pH drop earlier in the rigor process (Bendall, 1973). Therefore, beef and lamb are more susceptible to cold shortening than pork, which consists primarily of white muscle fibres (Savell *et al,* 2005).

According to Hannula and Puolanne (2004) as cited by Savell *et al* (2005) keeping the carcasses above 7 to 10°C before the onset of rigor will also have a positive effect on the aging process post mortem.

Heat shortening

Simmons (1997) and Uruh, (1986) discovered shortening of the fibres at high temperatures post mortem combined with a low pH (Thompson, 2002). This heat shortening or "rigor shortening" occurs when the muscles are subjected to high temperatures (above 15°C) for long periods post-mortem, leading to a more rapid glycolytic rate as a result of higher glycolytic enzyme activity and therefore a subsequent faster rate of pH decline, increasing the cellular calcium levels which then induces muscle fibre shortening (Lee & Ashmore, 1985). These conditions cause the proteolytic activity to decrease (Dransfield, 1993; Simmons, 1996) and drip loss to increase (Denhertogmeischke, 1997; Thompson, 2002).

This contraction/shortening of the muscle fibres occurs during the onset of rigor, since the release of calcium from the mitochondria will not occur when sufficient amounts of ATP are available. (Mickelson,

1983; Hwang *et al,* 2003) The contraction however is not as severe as that from cold shortening due to an insufficient amount of ATP to energize the contraction (Thompson *et al,* 2006).

The shortening of sarcomeres during rigor can never be completely prevented; however the degree of subsequent toughening can be reduced by implementing certain factors either environmental or physiological, before, throughout or after slaughtering (Savell *et al,* 2005).

3.3.4.3 Prevention of temperature induced shortening

Carcass weight and composition tend to play a huge role in the chilling rate (Savell *et al,* 2005). Adding to the carcass weight is subcutaneous fat (Dolezal, 1982). Smith *et al* (1976) showed that an increased subcutaneous fat thickness will allow the carcass to chill more slowly and increase the enzyme activity. This thicker fat layer either decreases the chilling rate of the carcasses by increasing insulation and/or by increasing the total mass of the carcass (as cited by Savell *et al,* 2005).

Today, the most common method of reducing fibre shortening is electrical stimulation. Simply by increasing the rate of pH decline it reduces the general rigor period, giving motive for using a faster chilling regime (Savell *et al,* 2005).

Electrical stimulation

Electrical stimulation was initially invented by Benjamin Franklin and from there evolved over the years to the system used today by the Australians (Bouton *et al,* 1978, 1980; Powell, 1991) and New Zealanders (Chrystall *et al,* 1983).

During electrical stimulation (ES) an electrical current is sent through the carcass of a freshly slaughtered animal. This current causes the muscles to contract which increases the glycolytic rate and in turn lowers the end pH. Therefore, with electrical stimulation, rigor mortis develops more rapidly and at higher temperatures which in turn prevent the muscles from excessive shortening and leads to increased aging rates (Davey & Gilbert, 1976; Savell *et al,* 1977b; Swatland, 1981; Marsh *et al,* 1987; Sorheim *et al,* 2001 as cited by Hwang *et al,* 2003).

It has also become apparent that a lower end pH was not the only effect that electrical stimulation has on the tenderising of meat. Devine *et al* (2002) evaluated 350 lamb carcasses that entered rigor all at the same temperature irrespective whether they were stimulated or not. The stimulated carcasses still had the highest rate of post mortem tenderisation. This illustrated that electrical stimulation not only

influenced the increased declining rate of the pH but other mechanisms as well. Therefore, three actions of electrical stimulation on the post-mortem muscle have been stipulated. These are the prevention of cold-induced shortening by ensuring that the muscle enters rigor at the optimal conditions (pH and temperature), the physical disruption of the muscle fibres and the acceleration of proteolysis (as cited by Hwang *et al,* 2003).

The rise in cytoplasm calcium concentration during electrical stimulation is different from cold shortening as the latter's increased calcium concentration comes from a reduced function of the sarcoplasmic reticulum. As a result of the earlier attainment of rigor mortis in stimulated muscles they will also experience a faster tenderisation due to entering rigor at a higher temperature (Hwang *et al,* 2003).

In the beginning, it was thought that to achieve the greatest effect from ES, the higher the voltage, the better. Therefore, peaks up to 1 130V were used in some New Zealand systems. It was also thought that low voltage systems (40V) worked effectively when implemented before the nervous system became inactive (Devine, 2001).

To clarify this argument Eilers *et al* (1996) compared the effects of three different ES methods to un-stimulated carcasses. All ES carcasses were divided into three groups. In the first ES group the carcasses were stimulated with 240V at 60Hz throughout the entire body by attaching a nose hook directly after exsanguination. Contraction of all major muscles was visible within this treatment. The second ES group was stimulated with 35V at 60Hz on the dressed, already split carcasses through four successive contact electrodes localized on the rib/short-loin region. This treatment had a more localised effect and very little contraction was observed in the round muscles of the carcass. As a result the pH decline was much more rapid in the LM than in the SM for this treatment. The third ES treatment was a combination of the first two treatments. From this Eilers *et al* (1996) reported that 67.2% of ES-III carcasses, 51.6% of the ES-II carcasses, 37.5% of the ES-I carcasses and 12.5% of the control carcasses had lower pH_3 values than 6.2 for the LM muscle at 3h post mortem. In the ES-III group 31.3% had pH_3 values of less than 6.0. Olsson, Hertzman and Tornberg (1994) also proved that using low voltage stimulation was not always as effective against shortening and toughening in beef muscles (As cited by Sorheim *et al,* 2001). Eilers *et al* (1996) also compared the average pH levels of all three ES methods and compared it to those from the non-stimulated carcasses. The information gained established that all the electrically stimulated groups had decreased ($P<0.01$) pH values for the LM and SM muscles 1.5 and 3 hours post mortem and that it was kept lower ($P<0.01$) than those from the non stimulated carcasses even up to 24 hours post mortem (Eilers *et al,* 1996).

Comparisons for tenderness were investigated in the same study by Eilers *et al* (1996) between the different ES methods and the unstimulated carcasses. Since no differences in shear force were found

between the three ES methods, they compared an average shear measurement of the three ES methods to that of unstimulated muscles. A significant increase (P<0.10) in panel tenderness and a decrease (P< 005) in shear force were revealed for stimulated LM muscles. The tenderness of the GM and SM muscles were found to be unaffected by ES (P> .05).

According to other studies (Marsh *et al,* 1987; Smulders *et al,* 1990; Pike *et al,* 1993) a lower frequency of ES is more effective than the high frequency (60Hz) used in the study of Eilers *et al* (1996). The differences between the rates of muscle pH declines on account of different frequencies of ES was due to the different reactions of the muscles to high (60Hz) and low (2-15Hz) electrical frequencies (Marsh *et al,* 1985). High frequency stimulation can lead to complete tetany of the muscle, whereas stimulating with low frequencies results in a series of small muscle twitches. In the case where a muscle resides in a state of tetany, less ATP is being utilised which slows down the glycolytic rate. Whereas, lower frequency stimulated muscles that undergo a series of contractions, uses more ATP, thus having a higher glycolytic rate (Eilers *et al,* 1996). Pike *et al* (1993) found an end pH_3 of 5.61 in *longissimus* muscles when beef carcasses were stimulated with 75V at 15Hz directly after exsanguination (Eilers *et al,* 1996).

As previously mentioned, an optimal rigor temperature of 15°C has been recommended for meat to achieve an optimum tenderness level (Hwang *et al,* 2003). Therefore the question arises to which extent ES should be implemented to achieve rigor at this optimum temperature.

Even though stimulated carcasses were still more tender than those which were not electrically stimulated, carcasses electrically stimulated within 2 minutes after slaughter produced meat that has been found to be less tender than that of carcasses stimulated 30 minutes post slaughter. Even after 14 days of ageing the meat stimulated 30 minutes post mortem were still the most tender (Whalgren *et al*, 1997; Hwang *et al,* 1998, 2003). Hwang and Thompson (2001a) studied these effects of applying stimulation either immediately after slaughter or just before entering the chiller. Measurements of the glycolytic rate, protease activity and additional meat quality traits showed that stimulation directly after slaughter caused a much faster pH decline and in turn lowered the μ-calpain and increased the calpastatin activities causing subsequent increased shear force values (Thompson, 2002). Hwang and Thompson (2001b) came to the conclusion in a following study that the rate of pH fall was the main effect influencing the tenderness of the meat during the period of aging where a rapid pH fall leads to a reduced ageing potential and increased drip loss. It was concluded that to achieve the most tender meat after 14 days of age, the pH decline must be at such a rate to reach a pH of 6.0 at temperatures between 29°C and 30°C. This meant that overstimulation at abattoirs will lead to heat shortening which, as mentioned, implies a pH of 6.0 at temperatures higher than 35°C (as cited by Thompson, 2002).

It was these studies that led the MSA to implement the pH/temperature window in production systems. With this they could maintain the electrical inputs to such a level that a pH/temperature relationship of greater than pH 6.0 for muscle temperatures greater than 35°C and a pH of less than 6.0 for muscle temperature less than 12°C could be obtained (Thompson, 2002).

Due to the importance and difficulty of implementing ES at the right time and at the right place for the right reasons, Simmons *et al* (2008) found it applicable to reassess the principles of electrical stimulation.

As mentioned, the main motivation for using ES was to eliminate the occurrence of cold shortening. This transpired due to early practices of freezing the entire lamb carcass for large scale exports. Contracture of the muscle fibres has been known to start from a temperature of 25ºC and becomes more intense as temperatures decrease (Davey & Gilbert, 1975; Honikel *et al*, 1983). For cold shortening to be applicable in the industry today, a significant shortening of 20% of the rest length of the muscle must be achieved during the chilling period until rigor mortis has set in. Through the knowledge that cold shortening requires ATP for the muscle fibres to contract, it leads to the conclusion that the extent of contraction reduces with the declination of the affected pH and comes to a stop at pH 6 (Scopes, 1971). From this it was once more made apparent that if temperatures were kept above 10ºC before the pH dropped to below 6.0, the incidence of cold induced shortening would be abridged.

To prove this theory Simmons *et al* (2008) built a model by using beef *longissimus* muscles at different pre-rigor pH levels and transferred them from a waterbath of 35ºC to one that was kept at 0ºC. Tenderness measurements were taken after the meat was aged for two weeks at a temperature of 0ºC. These results also demonstrated that cold induced shortening does not occur if the muscle pH is below 6 when the temperature of the meat reaches below 10ºC. The claim was made that in today's commercial slaughter process it is very unlikely that a carcass will reach 10ºC before a pH of 6 or lower is achieved, as it will require a very extreme chilling regime (Simmons *et al,* 2008).

Reducing variation

Heamshaw *et al* (1998) utilised the meat of steers containing different levels of *Bos indicus* over a aging period of 28 days, proving that by using ES these variances can be reduced immensely to obtain optimum tenderness levels and eating quality (Figure 3.2).

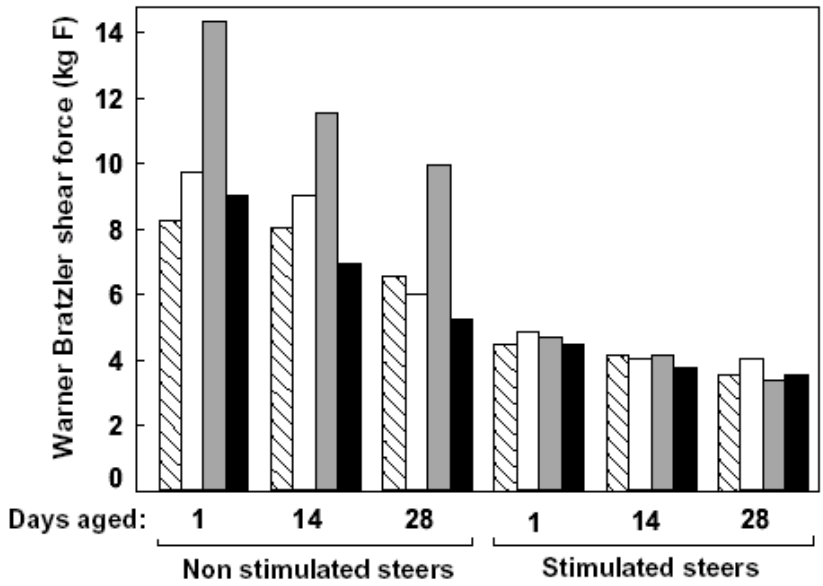


Figure 3.2. The effect of electrical stimulation and ageing duration on peak Warner Bratzler shear force of striploins from steers with varying Brahman content (0% Brahman (striped bars), 17 - 3% Brahman (open bars), 50% Brahman (shaded bars) and 67 - 100% Brahman (solid bars). Data adapted from Heamshaw *et al* (1998) by Devine (2001)

Chilling

Post mortem chilling exists for three basic reasons, being food safety, elongated shelf life and reduced shrinkage which in turn also increase the tenderness and affects the colour.

The common use of rapid carcass chilling and pre-rigor freezing became apparent in the 1960's and 1970's (Sorheim & Hildrum, 2002). Besides the fact that rapid chilling increased the capacity of the chillers, decreased the weight loss of the carcasses through minimising drip loss and improved the microbiological state of the carcass, the occurrences of cold shortening were still imminent (James & Baily, 1986).

Another method developed for decreasing the fibre contraction was the immediate post-slaughter freezing at a temperature of -30°C, followed by storage at -5 °C. Such conditions ensured the continuance of glycolysis, the depletion of ATP and therefore prevented shortening during thawing. (Moran, 1930; Smith, 1930; Marsh & Thompson, 1958) Further guidelines included the utilising of a specific time-temperature combination that guaranteed the freezing of sarcomeres in a pre-rigor, elongated state. Storage after freezing at -5°C resulted in more rapid ATP depletion than -10°C, provided that the duration of storage was sufficient to allow the pH to decrease below 5.8 consequently alleviating subsequent thaw rigor (Koohmaraie, 1996).

As a result of the various effects of pH, temperature, time and other environmental influences on meat tenderness mentioned above, new methods of improving the tenderness and decreasing the variance thereof between animals is being developed regularly. Some of these "new" methods include:

Calcium activated tenderisation (CAT)

Beef cuts have been injected with a calcium chloride solution increasing the rate and degree of proteolysis (Koohmaraie *et al,* 1995) The effectiveness of Calcium activated tenderisation has been demonstrated by a number of researchers (Koohmaraie *et al,* 1988, 1989, 1990; Koohmaraie & Shackelford, 1991; Wheeler *et al,* 1991, 1992, 1993, 1994; Kerth *et al,* 1995; Lansdell *et al,* 1995; Miller *et al,* 1995b*;* Wulf *et al,* 1996), nonetheless limited acceptance of this method has been seen by the meat industry.

Hydrodyne

This is where hydrodynamic shock waves, caused by a small explosion, tenderise the meat cuts which are submerged in water (Solomon *et al,* 1997; Calkins, 1997).

Blade or needle tenderisation

The physical tenderisation of meat cuts post mortem (Savelle *et al,* 1977a).

Sodium phosphate and sodium lactate

Injection of sodium phosphate and sodium lactate solutions used normally in pork to increase tenderness and flavour of fresh pork products (Vote *et al,* 2000).

Marinades

The infusion of beef muscles with a blend containing phosphates and lactates successfully enhance the sensory attributes of beef (Sacanga *et al,* 2000; Hoffman *et al,* 2008).

Further processing

This includes cubing, cooking, grinding, or flaking (Tatum *et al,* 1999).

Aging

Sufficient aging of meat is probably the easiest and most well known method of minimising the variance in meat tenderness. This method plays a vital role in every day meat production and is therefore rightfully added as the fourth CCP in a slaughter line. For reaching an adequate level of tenderness and the minimum variation, aging must be maintained for at least 10 to 14 days for beef (Table 2). This method became very popular with the invention of vacuum packaging (wet aging). Prior to the development of this technique, primal cuts were stored in a refrigerator (dry aging), however this method led to enhanced discolouration as well as drying out of the cuts (Koohmaraie, 1996).

Table 3.1. Effect of the length of post mortem storage on beef shear force values (Wheeler *et al,* 1996)

Breed	n	Mean	7 Days aging Range	% > 6kg	Mean	14 days aging Range	% > 6kg	Correlation Day 7 to Day 14
Angus	102	5.11	2.57 – 9.30	22	4.05	2.48 – 9.04	5	.58
Tuli	158	5.71	2.94 – 12.38	34	4.58	2.33 – 9.24	8	.65
Hereford	106	5.67	2.37 – 11.91	31	4.74	2.41 – 8.30	12	.66
Belgian Blue	144	5.82	2.52 – 10.57	42	4.82	2.64 – 8.41	14	.61
Boran	138	6.58	3.15 – 11.79	55	5.14	2.84 – 11.25	26	.76
Brahman	119	7.30	3.43 – 12.50	63	6.05	2.66 – 11.03	34	.80
All breeds	761	5.95	2.37 – 12.50	42	4.86	2.33 – 11.25	17	.72

(Adapted from Koohmaraie, 1996)

Stretching or restricting

Other methods to avoid the shortening of fibres is the physical stretching of the muscles or restricting them from shortening (Locker, 1960), by either laying the carcasses in a horizontal position or by hanging them on the pelvic bone (Herring *et al,* 1965; Hostetler *et al,* 1972; Bouton *et al,* 1973). A different method is the hot deboning of the primal muscles, stretching them pre rigor by packing them in a plastic casing that functions as an exoskeleton that stops shrinkage/contraction of the fibres (Devine *et al,* 1999). For the purpose of this study, these restricting or stretching methods is discussed in depth.

According to Koohmaraie (1996) the problem was that although the efficiency of all these methods was well documented, there was still a lack of implementation in the industry.

3.4. Alternative carcass suspension or pre-rigor stretching

As previously stated an optimal slaughter process involves the maximum extent of proteolysis, the minimum shortening or maximum stretching of the muscle post mortem and the genetic and environmental effects on the muscle structure response post mortem (Thompson *et al,* 2006).

During the late 1960s and early 1970s a great deal of research was conducted on numerous techniques for either stretching or restricting the contraction of muscles. (Sorheim & Hildrum, 2002) It was found that the degree of shortening is dependent on the temperature and the amount of tension placed on the muscle at rigor mortis (Locker & Hagyard 1963 as cited by Thompson *et al,* 2006).

Herring *et al* (1965b) showed that with conventional vertical carcass suspension on the Achilles tendon, some muscles increased and others decreased in tension. Through these findings it was still not certain whether to restrict a muscle from shortening or to stretch a muscle during the onset of rigor mortis for achieving the maximum tenderness level. Herring *et al* (1967) conducted a study specifically for this reason, where the effect of 48% shortening and 48% stretching of the pre-excised *semitendinosus* muscle on the tenderness level of the meat was examined. A significant ($P<0.01$) effect was found on tenderness and shear force values between treatments. It was said that with a decrease in sarcomere length of 50% there would be an increase in shear force of almost double the original amount. Between 10% and 50% stretching however, no significant differences were found for shear force as the percentage extension increased (Herring *et al,* 1967; Sorheim & Hildrum, 2002).

Other studies have measured up to a 20% decrease in the excised *sternomandibularis* muscle length however, still no significant decrease in tenderness. Tenderness however decreased readily above 20% shortening, peaked at 40% shortening and increased again above 40% shortening. In fact they discovered that muscles that shortened 55-60% had the same WBSF values as those that have shortened less than 20% of its original length. (Herring *et al,* 1967) Therefore Marsh and Leet (1966) stipulated that with a shortening of 20% to 40% definite toughening of the meat will be observed (Herring *et al,* 1967). The difference in tenderness of muscles stretched 12% to 48% although apparent, was not of the magnitude that it was evident with the various stages of shortening (Sorheim & Hildrum, 2002). Similar results were established by Simmons (1999) (as cited by Sorheim & Hildrum, 2002) when *longissimus thoracis* muscles were stretched to 20, 40 and 60% of its original pre-rigor length. Only the

20% stretching reduced the shear force measurement whereas no significant difference was found for increasing the length to 40% and 60%.

From the basis of these results, it was apparent that it was more important, from the standpoint of ultimate tenderness, to prevent post mortem shortening than to ensure maximal stretch. (Herring *et al,* 1967) This was in accordance to the research by Koohmaraie (1996) where the sarcomere length and subsequent tenderness only had a significant correlation in the pre-rigor state also leading to the conclusion that muscle fibres should be restricted from shortening during rigor rather than being stretched to its maximum capacity.

The mechanism by which stretching the muscle increases tenderness is due to the effect it has on both the myofibrils and the connective matrix (Bouton *et al,* 1973; Hostetler *et al,* 1972; Thompson, 2002). Herring *et al* (1967) studied the effect of animal age on sarcomere length and found that they were highly significant. Excised muscles were evaluated in either a stretched or contracted state over two different animal age groups. Although all the levels of stretched muscles over both age groups showed a significant increase in tenderness and sarcomere lengths over the contracted muscles the sarcomeres in the older group were always shorter than those from the younger group.

Increased tenderness obtained by stretching could, however, be positively influenced by the changes in connective tissue (Bouton & Harris, 1972). Contracted muscles have higher adhesion values than stretched muscles, which prove that the tenderness attained by aging is influenced by the fibre contraction state of the muscle. The contracted muscle fibre greatly affected the connective tissue of the muscle and therefore gives reason for its lower tenderness level than stretched muscles after an aging period (Bouton & Harris, 1972). Shortened muscles however, even after 10 days of aging, were not acceptable in tenderness and were most apparent in older animals (Herring *et al,* 1967).

In addition to all the benefits documented on these methods, it wasn't until the late 1990s when some of these practices were implemented in the meat industry, mainly as a result of an increased demand for improved meat tenderness from large food store chains. Countries that have implemented these methods include the United Kingdom, Ireland, Australia, New Zealand, Sweden and Norway (Sorheim & Hildrum, 2002).

3.4.1 Restriction or stretching of individual muscles.

Hot-boning

Hot-boning is the pre-rigor excise of muscles or muscle systems directly after slaughter while the muscles are still in their uncontracted, pre-rigor state. This procedure was a result of increased pressure from the industry for a decrease in energy usage and increased requirements for chiller space.

Benefits from using hot-boning have proved to be very economical. Results from hot-boning muscles have shown reduced weight loss, decreased drip loss, lower chilling costs and higher water binding capacity of the meat (Pisula & Tuburcy, 1996). An intact carcass can lose up to 2% weight through evaporation when chilled. Drip losses during storage while vacuum packed is reduced by 0.1% to 0.6% depending on the type of muscle and the chilling regime it is subjected to. On account of hot-boned meat having higher water holding capacity and better fat emulsion properties it is well suited for use in comminuted meat products such as sausages (Hamm, 1982). Further advantages of using hot-boning is an increase of between 50-55% of storage space when individual muscles are packed in boxes compared to whole carcass chilling (Pisula & Tuburcy, 1996). It was also easier to create an optimum chilling-regime for individual muscles than for those still connected to the intact carcass (Sorheim & Hildrum, 2002). In turn this method saved refrigeration energy and transport costs.

The problem with hot-boning, not to mention high initial construction and staff training costs, is that by excising these muscles from the carcass the natural restriction from the skeleton and connective tissue was lost, which increases cold induced shortening during the chilling period (Sorheim & Hildrum, 2002).

For this reason some mechanical fixation or stretching techniques were researched. Herring *et al,* (1965a) expanded this research and allowed excised muscles to shorten while undergoing rigor mortis. After cooking these muscles, those that were restrained from shortening were more tender than those unrestricted (Herring *et al,* 1967). Stouffer *et al* (1971) patented a pre-rigor method of tenderising muscles by applying tension with weights or a mechanical device designed for this purpose. One of the devices consisted of a number of pins attached to each end of an adjustable bar. The pins were inserted directly into the muscle of interest and were anchored at another point, allowing extension of the pre-rigor muscle. Bruce and Ball (1990), Beuge and Stouffer (1974) and Herring *et al* (1967) conducted further studies where the individual muscles were either glued or clamped and then stretched to a certain length (Sorheim & Hildrum, 2002). Although all these methods of tensioning reduced shear force values, they

required a large amount of time and equipment and are therefore not currently used in the industry (Ludwig *et al,* 1997).

Wrapping

Wrapping is a different procedure designed for restricting the contraction of hot-boned muscles. Devine *et al* (1999) excised and wrapped beef *longissimus* muscles in a cling polyethylene film at temperatures of 20 to 35ºC before the onset of rigor at temperatures below 10 ºC and found that it reduced the sarcomere shortening and increased the muscle tenderness compared to the unwrapped muscles. Conversely the muscles that entered rigor mortis at a temperature of 15ºC were unchanged by wrapping.

In a similar study beef *longissimus* muscles were removed and wrapped and allowed to enter rigor at 4ºC so as to encourage cold shortening. Results showed higher tenderness scores in wrapped muscles than in unwrapped muscles except for those entering rigor at 12ºC where no difference occurred. For the same study *semimembranosus* muscles were wrapped at rigor temperatures of 4ºC and 12ºC and no subsequent increase in tenderness were found at either temperature. It was thought that due to the shape and size of this muscle, it was more difficult to inhibit the fibre contraction (Hildrum *et al,* 2000).

Since then, improved wrapping methods have been introduced such as the Pi-Vac Elasto-Pack system (Meixner & Karnitzschky, 2001). This system involves a packing chamber through which a highly elastic film is stretched to the inside walls of the chamber. The pressure is released after the muscle is inserted into the chamber and the film returns to its original size, obstructing the diametrical extension, which occurs when the muscle shortens in length, therefore inhibiting the shortening of the muscle.

Studies with this system showed an improved tenderness in *longissimus* muscles when wrapped before rigor for both rigor temperatures of 4ºC and 14ºC. This proved that this method had some advantages which included the ability to rapidly chill these muscles without any negative effect on meat tenderness (Hildrum *et al,* 2001) and to achieve a more attractive shape for meat cuts (Hildrum *et al,* 2002). Unfortunately some Pi-Vac Elasto-Pack systems are non-continuing systems which are very labour intensive, although research is being done to improve and incorporate these systems into high-speed production lines (Meixner & Karnitzschky, 2001).

3.4.2 Intact muscle stretching

During the 1960s and early 1970s more emphasis was placed on increasing the tenderness of intact carcass muscles by means of stretching through the use of different hanging positions of the carcass. It

was said that the extent of intact muscle shortening during chilling has its own physical restriction due to the muscles being connected to the skeleton and connective tissue (Hostetler *et al,* 1970).

Herring *et al* (1965) evaluated the differences in tenderness between muscles from carcasses being placed in different hanging positions whilst entering rigor. A horizontally placed side with limbs hanging free showed an increase in sarcomere lengths, smaller fibre diameters and an increased tenderness level for the *Longissimus, Gluteus medius, Biceps femoris* and *Semitendinosus* muscles compared to muscles from carcasses suspended on the conventional Achilles tendon. By the 1970s, a method called "Tenderstretch" was introduced by Hostetler *et al* (1970). The Texas A & M Tenderstretch tenderising method involves the suspension of beef carcasses through the eye of the aitchbone or the *obturator foramen*.

Hostetler *et al* (1972) tested this method of carcass suspension to reduce the sarcomere shortening. They studied many different suspension methods and found that this hip-free method, suspended from the obturator *foramen*, was the most effective in increasing the sarcomere length and decreasing the shear force values of the loin and round muscles. Hostetler *et al* (1975) found 17% longer sarcomeres and less overlap of thick and thin filaments from using Tenderstretch (Savell *et al,* 2005).

This method involves placing an S-shaped hook in the eye of the aitchbone (*obturator foramen)* as an alternative to the conventional Achilles tendon position (Figure 3.3) (Harris, 1974). This allows the hind leg to hang free at a 90° angle towards the vertebrae. This procedure should be implemented between 45-90 minutes after bleeding to ensure that the muscles are still in their pre-rigor state and still adequately extensible to allow the full effect of the stretch to occur (Sørheim & Hildrum, 2002).

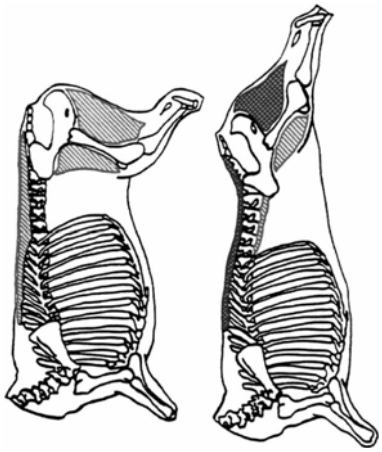


Figure 3.3. Schematic drawings of the Tenderstretch method (left) and the Achilles tendon method (right) showing the different muscles affected (Harris, 1974) Hatched areas – relaxed and tender muscles. Darkcross-hatched areas – contracted and tough muscles

Tenderstretch or pelvic suspension either stretches or prevents the muscle from shortening during the rigor process by increasing the tension on certain muscles of the hindquarter and subsequently improving its tenderness (Hostetler *et al,* 1972; Bouton *et al,* 1973; O'Halloran *et al,* 1998; Thompson, 2002).

Pelvic suspension (Hostetler *et al,* 1972) and skeletal alteration to permit muscle stretching (Wang *et al,* 1994; Ludwig *et al,* 1997) have been introduced as the third CCP as a precaution to early post mortem toughening.

3.5 Effect of Tenderstretch

The mechanism of stretching the muscle to prevent pre-rigor shortening and improve the eating quality of the meat is quite complex (Thompson *et al,* 2006). Possible mechanisms causing the decrease in tenderness from cold shortening includes the degree of overlapping of the actin and myosin (Marsh & Carse, 1974), changes in the gap filaments (Locker, 1982), changes in collagen orientation (Rowe, 1974; Purslow, 1999) or changes in the roles of both the myofibril and connective tissue components (Hostetler *et al,* 1970; Bouton *et al,* 1973).

Hopkins and Thompson (2001) studied the amount of energy needed to break the actomyosin complex in carcasses subjected to different stretching treatments thus containing different degrees of overlapping between the actin and myosin filaments (cited from Thompson *et al,* 2006). It was shown that even though stretching increased sarcomere length and reduced the overlap between actin and myosin there were no correlation between the degree of actin and myosin overlap and the amount of energy required to dissociate them. Therefore, it was more likely that the subsequent increased palatability was not from the breakage of actin-myosin bonds but probably from an increased rate of degradation of the structural proteins at the Z-disk junction and intermyofibre filaments (Hopkins & Thompson 2001; Thompson, 2002; Thompson *et al,* 2006).

From the study of Marsh (1966) and Marsh and Leet (1966) it became clear that "background toughness" might affect the contraction state of the muscle and its subsequent tenderness, thus two age groups (young and old) were included in the study of Herring *et al* (1967) where a significant ($P< 0.01$) effect of age was found on the shear force and the panel tenderness values for stretched and unstretched muscles, possibly as a result of the higher percentage perimysium in the old age bovine group.

3.5.1 Sarcomere length

Elongated sarcomere lengths were found by Hostetler *et al* (1970, 1972) in *Longissimus, Semimembranosus,* and *Semitendinosus* muscles when using Tenderstretch. No change however was documented for the *psoas major* muscles, probably as a result of decreased tension due to the aitch bone suspension method (Sorheim & Hildrum, 2002). Besides the increased sarcomere lengths of M. *Semimembranosus* ($P<0.001$) acquired from the Tenderstretch method, a decrease in the in-sample variation from 11.3% to 6.5% was also detected (Ahnstrom *et al,* 2005). From studying the effects of Tenderstretch on young and older animals, older animals had mean sarcomere lengths of 1.83 ± 0.05 µm and 2.54 ± 0.05 µm for the control and aitchbone hung *Semimembranosus* muscles respectively, whilst 1.89 ± 0.05 µm and 2.11 ± 0.05 µm were measured for normally hung and stretched *Longissimus dorsi* muscles. Similar results were shown for younger animals where the *Semimembranosus* muscles had sarcomere lengths of 1.80 ± 0.07 and 2.88 ± 0.07 for the control and stretched carcasses respectively, and lengths of 2.07 ± 0.03 and 2.28 ± 0.03µm were obtained for the respective *Longissimus dorsi* muscles (Bouton & Harris, 1972).

3.5.2 Shear force

Hostetler *et al* (1972) and Bouton *et al* (1973) found a highly significant interaction ($P<0.001$) between hanging method and the eating quality of the meat. Hostetler *et al* (1970, 1972) found that Tenderstretch decreased the shear force values for the *Longissimus* and *Semimembranosus* muscles but not for the *Semitendinosus* muscle where only increased sarcomere lengths were observed without a decrease in shear force (Sorheim & Hildrum, 2002). Bouton *et al* (1973) also reported decreased shear force for the *Gluteus medius* muscle. In this study the Tenderstretched meat was found to have unaged meat tenderness values equivalent to that found after 21 days of aging. Muscles with high connective tissue levels were least affected by the Tenderstretch method and showed a decrease in tenderness (Ferguson *et al,* 1999).

The *Biceps femoris* and the *Semitendinosus* muscles were the least affected by the Tenderstretch method in the study of Joseph and Connoly (1976), where the high connective tissue and sinew content of the *Semitendinosus* muscle had a more substantial effect on tenderness than the subsequent stretching of the myofibrils. It is apparent through former data that a shearing force through a unit area of stretched muscle must cut more fibres, more endomysium and more perimysium than a similar shear through contracted muscle. This questions the possibility of changes in muscle connective tissue in regard to drastic changes in muscle length (Herring *et al,* 1967).

Studies by Furguson *et al,* (1999) showed an increase in palatability for most of the hindquarter muscles except for the *M. psoas major* which experienced less tension during the Tenderstretch method than the normally hung sides. In the *M. Semitendinosus* or “eye round” no differences in palatability were noticed because of the fact that the same amount of stretching were found in both Tenderstretch and normally hung carcasses. All the carcasses in this study were electrically stimulated and still a significant effect of Tenderstretch were observed in some of the hindquarter muscles (Table 3) which could portray an additive effect of these procedures on palatability (Thompson, 2002).

Table 3.2. Palatability scores for muscles from ES tenderstretched and achilles hung sides after adjustment for cooking, hanging, US marbling and ossification scores and their interactions (Ferguson *et al,* 1999)

Primal cut	Muscle	Tenderstretch	Achilles tendon	Significance
	Forequarter			
Brisket	*Pectoralis profundus*	32	35	ns[a]
Blade	*Triceps brachii*	55	56	ns
Oyster blade	*Infraspinatous*	61	62	ns
Cube roll	*Longissimus thoracis*	65	63	P<0.05
	Spinalis dorsi	75	76	ns
	Hindquarter			
Striploin	*Longissimus lumborum*	61	55	P<0.001
Tenderloin	*Psoas major*	71	74	P<0.01
Rump	*Gluteus medius*	64	57	P<0.001
Topside	*Semimembranosus*	45	38	P<0.001
Outside flat	*Biceps femoris*	50	47	P<0.001
Eye round	*Semitendinosus*	48	47	ns
Knuckle	*Rectus femoris*	50	48	P<0.001

[a] ns, Not significant

3.5.3. Variance

Sorheim *et al* (2001) showed a lowering in variation of tenderness along the length of the *longissimus* muscle rather than an overall increased average in tenderness level. Although the effect of Tenderstretch differed between carcasses with some improving a substantial amount and some very little, those with the greatest gains was found to be the carcasses with the least palatability scores for the normally hung sides. Thompson *et al* (2002) measured a decrease in variance of almost 25% between the palatability of Tenderstretch and Achilles tendon suspension methods (Thompson, 2002).

The variance in Tenderstretch sides of the corresponding carcass was about 50% less than that of the normally Achilles hung sides. A study done by the MSA found that tenderstretched sides had a variance of 9 palatability units whereas normally hung sides varied around 12 palatability units (Thompson, 2002). These results were again confirmed by Thompson *et al* (2005) when the carcasses of Tenderstretch and Tendercut were evaluated. Ahnstrom *et al* (2005) tested the effects of Tenderstretch and Achilles hung carcasses on the *M. Semimembranosus* muscle. A significant decrease in variation of shear force was found within the treatment. Tenderstretch carcasses had a variation coefficient of 12% compared to the 26% variation in shear force for the normal Achilles hung carcasses. Even within the sample there was lower variation in shear force for Tenderstretch treatments (10%) than for the normally hung carcasses (13.3%) ($P<0.001$) with a standard deviation within the samples of 3.9-20.7 for Achilles hung carcasses and 2.7-8.2 for the Tenderstretch suspended carcasses. Although the average improvement of this pelvic suspension method above the normally hung was 14.5 N, the highest difference was 64.5N (Figure 3.4) (Ahnstrom *et al,* 2005).

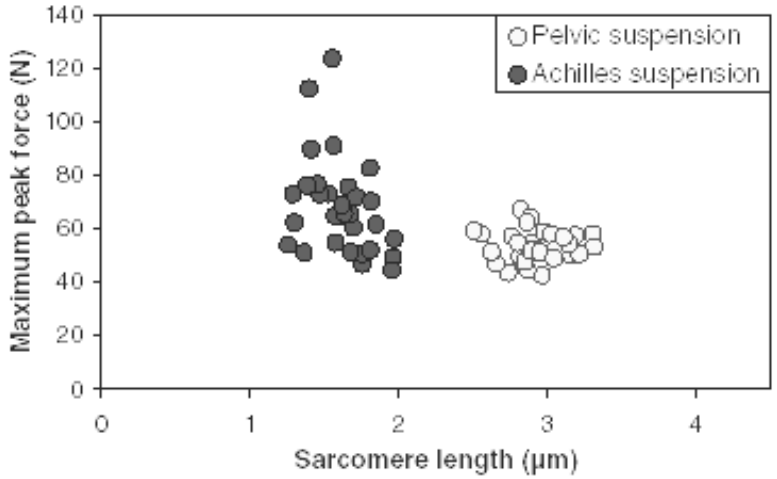


Figure 3.4. The effect of pelvic or Achilles tendon suspension on the variation between samples in shear force and sarcomere length in M. *Semimebranosus* (Ahnstrom *et al,* 2005)

3.5.4 Environmental effects on pre-rigor stretching

Rigor temperature

Devine *et al* (1996) and Locker and Hagyard (1963) reported that the optimal temperature at rigor for the highest tenderness score for normally hung sides is between 15°C and 18°C (cited by Thompson *et al,* 2006). A curvilinear relationship between tenderness score and temperature at rigor (pH 6.0), where the highest tenderness level was plotted at temperature 21°C and the lowest tenderness scores at both temperatures 4°C and 35°C, showed that in normally hung sides both the highest and lowest extreme

temperatures during rigor should be avoided for maximum eating satisfaction (cited by Thompson *et al,* 2006). For the Tenderstretched carcasses, no significant relationships were found between the rigor temperature and the tenderness score, thus making Tenderstretch a method that ensures tenderness either in extreme high or low temperatures (Figure 3.5) (Thompson *et al,* 2005).

This furthermore underlines the importance of processing carcasses at the correct temperature at rigor, for there was very little difference in tenderness scores between Tenderstretch and normally hung carcasses when the carcass reached rigor mortis at an optimal temperature such as 20°C. Although this could be an argument against the use of Tenderstretch, it is still very difficult for up to date processing methods to obtain the optimal processing conditions due to certain carcass factors such as glycogen content, carcass weight, carcass fat and whether the muscles are deep or superficial, not to mention the environmental effects between abattoirs and days (Thompson *et al,* 2006).

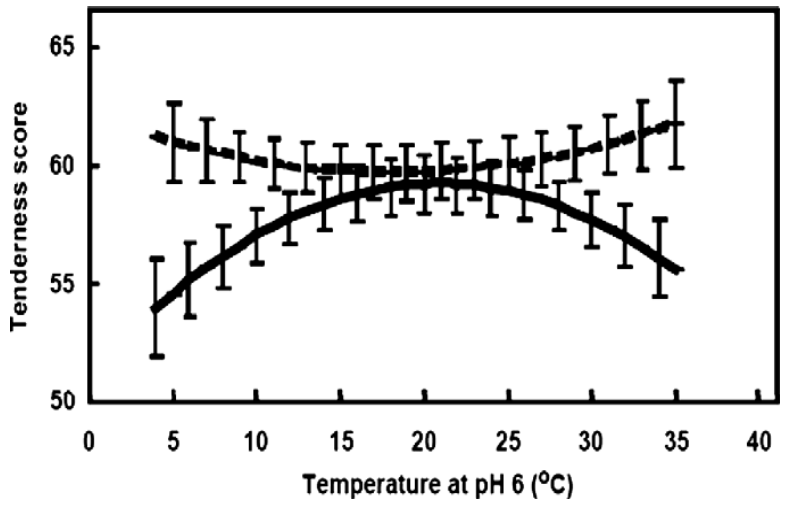


Figure 3.5. The relationship between tenderness score as a function of temperature at pH 6 in sheep carcasses. The solid line represents the predicted response for achilles hung carcasses whilst the dashed line represents the predicted response for the Tenderstretch carcasses. Data were adjusted for muscle, age category and post-mortem ageing time. pH and temperature were measured in the posterior portion of the M. *longissimus dorsi*. The vertical bars represent the standard errors of the predicted values. Adapted from Thompson *et al* (2005)

Chilling

A number of studies support the positive effects from using Tenderstretch on meat tenderness of beef, lamb and pork when these are rapidly chilled and have the risk of cold shortening (Bouton *et al,* 1973; Hostetler *et al,* 1975; Joseph & Connolly, 1977; Dreyer *et al,* 1979; Sorheim *et al,* 2001; Sorheim & Hildrum, 2002; Thompson, 2002; Thompson *et al,* 2006) and the lack of effect seen during medium

chilling rates (Dreyer *et al,* 1979; Sorheim *et al,* 2001). Thompson (2002) found the largest improvement in tenderness for Tenderstretch when carcasses were subjected to rapid chilling regimes where most of the normally hung sides had muscles that cold shortened.

This was an important discovery due to the fact that LD's of the carcasses used in the fast chilling experiments were all expected to suffer from cold shortening due to its location close to the surface of the carcass and its exposure to faster temperature declines (Locker & Hagyard, 1963; Olsson *et al,* 1994; Sorheim *et al,* 2001). Olsson *et al* (1994) found maximum muscle shortening in hot-boned LD's to be approximately 38% of their original length at rigor temperatures of 4°C. However, at rigor temperatures of 10°C only a 9% shortening was observed. Shear force measurements were 17.1 kP and 10.1 kP for the 4°C and 10°C rigor temperatures respectively, after 8 days of aging (cited by Sorheim *et al,* 2001).

Using Tenderstretch while rapidly chilling the carcass increased the sarcomere lengths from 1.64 to 2.03 µm and decreased the WB shear force from 103.2 to 61.4 N/cm^2 for the *Longissimus* muscle (Sorheim *et al,* 2001). Similar results were found in other studies such as increased sarcomere length from 1.9 to 2.3 µm and reduced shear force values from 6.0kg to 4.9kg at a 2 °C air temperature (Hostetler *et al*, 1972) whilst Bouton *et al* (1973) found longer sarcomere lengths ranging from 1.8 to 2.0 µm and lower shear force values ranging from 11.1kg to 5.7kg for Tenderstretch carcasses at a temperature of 2 °C (Sorheim *et al,* 2001). Dreyer *et al* (1979) compared Tenderstretch to normally Achilles hung carcasses at two different rigor temperatures (3°C and 9°C). Tenderstretched carcasses had longer *Longissimus thoracis* sarcomeres (2.6 µm - 1.9 µm) and lower shear force values (6.9kg - 10.3kg) than the normally hung carcasses at a low chilling temperature of 3°C, however at an air temperature of 9°C, regardless of the still lengthened sarcomeres from Tenderstretched carcasses, there were no difference in tenderness between the hanging methods (as cited by Sorheim *et al,* 2001).

It was therefore evident that this stretching or restricting of the muscles fibres from contraction counteracts the toughening of the meat during low rigor temperatures since WB shear force, sensory tenderness and hardness scores for the stretched muscles were found to only improve significantly when fast chilling was applied (Sorheim *et al,* 2001).

As mentioned before, the carcass factors responsible for the variation in rigor temperature such as whether the muscle is deep or superficial, glycogen content, carcass weight and fatness level and environmental effects such as the differences between abattoirs and different days of slaughter, brings out the true potential of Tenderstretch since it reduces these variations to produce overall tender meat (Thompson *et al,* 2006).

Genetic effects

Johnson and Thompson (2006) slaughtered Brahman steers and tropically adapted composite cattle genotypes where one side of each breed were tenderstretched and the other normally hung on the Achilles tendon. Results showed that in the normally hung and the Tenderstretch sides the Brahman breed had higher shear force measurements, although the differences between breeds became less evident in the tenderstretched sides. Thus genetic and phenotypic variance was a great deal smaller for tenderstretched muscle than for normally hung sides. Tenderstretch can therefore reduce the effect that genotype has on the tenderness (Thompson *et al,* 2006).

Electrical stimulation

Supporting the findings of Bouton *et al* (1978), Thompson *et al* (1999) also discovered that electrical stimulation has an additive effect to Tenderstretch on the eating quality of meat. Cooking losses and juiciness scores were higher in Tenderstretched sides (Bouton *et al,* 1973) and lower in ES sides relative to the control sides (Bouton *et al,* 1980). For this reason Tenderstretch may help alleviate the decreased juiciness of electrically stimulated muscle whilst ES will guarantee less cooking losses. Bouton *et al,* (1973) also found that unlike ES, muscles that have been Tenderstretched, have reduced adhesion strength between the fibres thus lessening the effect of connective tissue on the tenderness (Ferguson *et al,* 1999).

In addition, recent studies that have tested this interaction between ES and Tenderstretch have showed a decrease in thawing loss for both methods compared to their controls. A 0.95% decrease was found between ES and NES carcasses and a 0.71% decrease between Tenderstretch and the Achilles hung carcasses. However, when using ES and Tenderstretch in combination, ES lowered the thawing loss in both the hanging methods, although this was not significant ($P<0.397$). No differences were found with any combination for the values of cooking, drip and evaporation losses (Derbyshire *et al,* 2007).

Comparing shear force measurements between Tenderstretch and Achilles hung carcasses in groups that were either ES or NES, showed only a significant ($P<0.05$) decrease in shear force of 1.66kg for Tendertretch carcasses that were NES. Within the ES group, a non significant decrease of 0.28 kg was documented (Derbyshire *et al,* 2007).

These studies showed that although a hip suspended carcass had a significantly ($P< 0.05$) more tender *Longissimus* muscle compared to that of the Achilles hung carcass, the effect that ES or 7 days of aging or the combination of ES and 7 days of aging had on the hip suspended carcass was of no

significant improvement. This concurred with the results of Dransfield *et al* (1991) which found no significant benefit from combining ES with Tenderstretch (Derbyshire *et al,* 2007).

Aging

According to Herring *et al* (1967) aging of the meat had a greater effect on the normally hung muscles than on the stretched muscles from the Tenderstretched sides (Herring *et al,* 1967). Dransfield *et al* (1991) reported and Derbyshire *et al* (2007) later supported these findings that the combination of aging and hip suspension was superior to the effect of aging and the normal Achilles tendon suspension.

Davey *et al* (1967) found that the level of tenderness achieved by aging was strongly affected by the amount of myofibrillar contraction. It was found that at a 40% contraction, aging had almost no effect on the tenderness level (Herring *et al,* 1967). Therefore the combining of Tenderstretch and aging presented a positive effect on the palatability of striploin, outside round and rump muscles. However the aging rate was slower for the Tenderstretched muscles than from normally hung muscles (Bouton *et al,* 1973; O'Halloran *et al,* 1998). Steaks from the Tenderstretch sides were still more palatable in the early stages of aging. With the slower and faster aging rates for Tenderstretch and normally hung sides respectively, the palatability values of the meat cuts should converge with the ongoing aging period. After 5 d of aging, the aging rates of hindquarter and loin muscles from Tenderstretch sides were roughly 66% of that from the normally hung sides (Thompson, 2002).

In the study of O'Halloran *et al* (1998), it was shown that Tenderstretched meat has an initial lower shear force value than normally hung carcasses when the aging process begins. This could in fact be greatly beneficial for commercial conditions such as reducing the turnaround time of the production plant (Figure 3.6). A study from Bouton and Harris (1972) noted that, by implementing Tenderstretch, the tenderness in the *Longissimus dorsi* and *Semimembranosus* muscles at 2½ days post slaughter were equal to the tenderness of non-stretched muscles after 2 weeks of aging (Bouton & Harris, 1972).

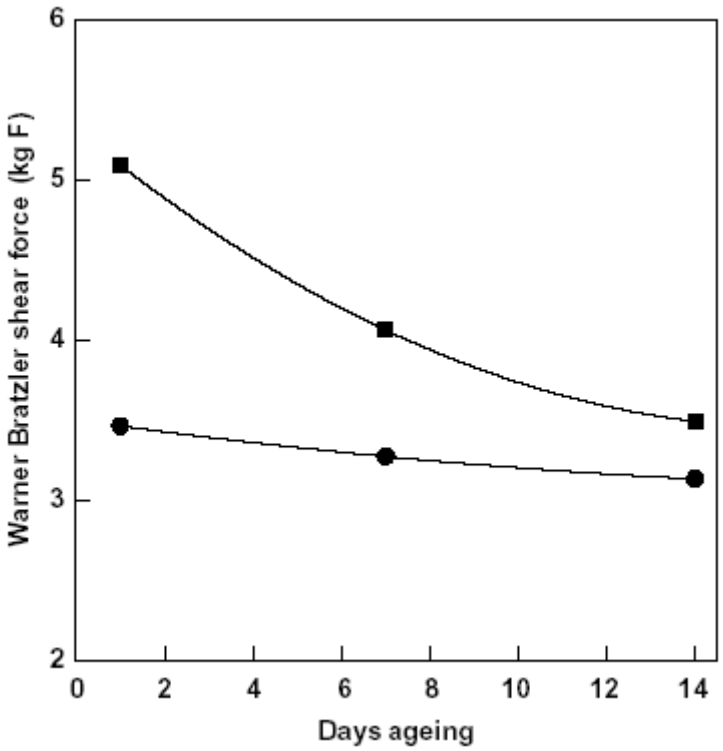


Figure 3.6. The changes in shear force versus days of ageing for Tenderstretch (circles) and normally hung Achilles suspended animals (squares). Data are from O'Halloran (1998) as reported by Devine (2001).

Cooking temperature

Various studies showed that the extent to which pre-rigor stretching influences the meat quality depends on the type of muscle and the temperature at which the samples are being cooked (Bouton *et al,* 1975; Bouton *et al,* 1978; Moller *et al,* 1987; Eikelenboom *et al,* 1998; Eikelenboom *et al,* 1998).

In the study of Giles (1969) the change in shear force due to the effects of aging and stretching were more distinct when the meat was cooked at 80°C where the thermal contraction was greater than at 60°C (Bouton & Harris, 1972).

Eikelenboom *et al* (1998) stated that high connective tissue muscle had a higher shear force from stretched carcasses than from normally hung carcasses when the samples were raw or cooked to a low internal temperature. Results showed that by cooking the *M. longissimus* muscle at low temperatures of between 55°C and 60°C the normal Achilles hung carcass had lower maximum shear force values. The shear force for Tenderstretch carcasses only started to decrease below that of the Achilles hung carcasses when the muscles were cooked at 80°C (Figure 3.7) (Thompson *et al,* 2006).

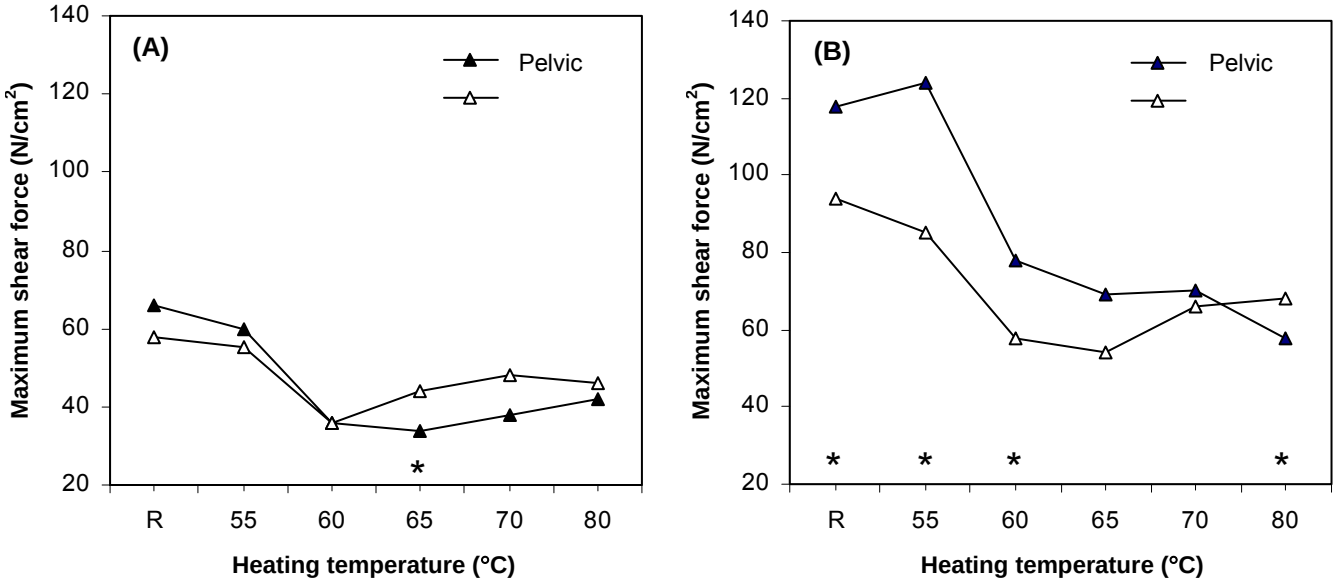


Figure 3.7. Warner Bratzler shear force values (N/cm^2) of the *Longissimus* (A) and *Semimembranosus* (B) muscles obtained from the pelvic (filled symbols) and Achilles tendon (open symbols) suspended sides. Each sample were tested when raw (R) and when cooked at 55, 60, 65, 70 and 80ºC for 1h. Significant differences between two methods of suspension (P<0.05) is marked with an apteryx (adapted from Eikelenboom *et al,* 1998).

Lepetit and Culioli (1994) revealed that in raw or low temperature cooked aged meat it was the collagen resistance and not the myofibre resistance that influences the maximal shear force value. Due to Tenderstretch and its subsequent stretching of the muscle fibres, it could be possible that the direction of the collagen fibres vs. the shear force plane has changed. As Rowe mentioned in 1974, the collagen fibres partly unfolds when the muscle is stretched pre-rigor and therefore is more oriented in the direction of the fibres, which means more collagen fibres being cut in the perpendicular shearing of the muscle fibres.

It is when the collagen denatures that its resistance decreases and the positive effects of Tenderstretch are experienced. These beneficial results occur at different cooking temperatures depending on the type of muscle, its collagen properties and the extent to which Tenderstretch stretches that specific muscle (Eikelenboom *et al,* 1998).

According to Tornberg (1996) raw meat is tougher due to a smaller viscous component within the muscle structure and the connective tissue starts to contract at a cooking temperature of 60°C and above. In the case of stretched muscle the extracellular spaces are smaller and therefore restrict the contraction of the connective tissue above 60°C, consequently producing more tender meat.

Water holding capacity

Ahnstrom *et al* (2005) found a definite increase in water holding capacity in the *M. Semimembranosus* by using the Tenderstretch suspension method. Results of their study showed a 2.4% unit decrease in the total amount of purge and cooking loss for the pelvic suspended carcasses. Eikelenboom *et al* (1998) also revealed a reduction in cooking loss for the same muscle when cooked at temperatures higher than 60°C.

3.5.5 Different Tenderstretch methods

Hwang *et al* (2002) (as cited by Thompson, 2002) considered two different methods of applying Tenderstretch, one the carcass being hooked at the aitch bone (*obturator foramen*) and the other through the pelvic ligament. This difference in the suspension hinge allowed for different tension strengths on different individual muscles. Most of the major muscles in the loin and hind leg experienced higher tension for both methods compared to normally hung carcasses. However, some smaller muscles differed in effect between methods and showed different degrees of shortening.

The correlation between sarcomere length and palatability were found to be very variable, some shortened muscles did not experience toughening (*M. gluteus profundus*) and some muscles stretched to lengths even greater than 2.0µm toughened (*M. tensor fascia latae*) (Thompson, 2002).

This proved that the correlation of shortening and palatability was inconsistent between different muscles. The variation between the anterior and posterior portion of the striploin in normally hung sides, however, was lessened with both methods of Tenderstretch. (Thompson, 2002).

With ligament suspension, longer sarcomere lengths in the larger hind leg muscles were measured than in those from aitch bone suspension, however no differences were found in shear force and palatability score. Overall, the hindquarter and loin muscles from the aitch bone suspended carcasses were 3.2 units more palatable than those that were suspended by the Achilles tendon (Thompson, 2002).

Tenderstretch proved to be a cheap and effective way of managing beef palatability and tenderness. There were some disadvantages in using the Tenderstretch method such as increased labour, for repositioning the carcass on and off the aitch bone before de-boning, and a decreased chiller capacity. According to Thompson (2002) these shortcomings seemed to be a small price to pay for increased palatability and tenderness.

3.6 Effect of Tendercut

Scientists at the Virginia Polytechnic Institute and State University established Tendercut (TC) as a new stretching or restraining method of major muscles in the carcass in the early 1990's (Wang, Claus & Marriott, 1994). Wang *et al* (1994) investigated the effects of cutting bone and connective tissues at the round/loin junction of pre-rigor beef carcasses on the tenderness levels of the meat. Researchers continued to study these effects of pre-rigor skeletal cuts for the improving of beef tenderness (Cotroneo, 1992; Wang *et al,* 1994*,* 1996; Claus *et al,* 1997; Ludwig *et al,* 1997; Beaty *et al,* 1999; Shanks *et al,* 2002). By using the TC method, tenderness improvements were found in the round and sirloin muscles and especially in the *longissimus* muscle (Shanks *et al,* 2002).

This procedure requires making cuts in the bones of a pre-rigor carcass while suspended by the conventional Achilles tendon (Figure 3.8) (Claus, 2002). Two selected positions in the vertebrae are cut, severing the bone, connective tissue, adipose tissue and some minor muscles. The weight of the forequarter is therefore suspended just on the *Longissimus* muscle and some round muscles.

The first cut is applied at the normal separation point of the hind and forequarter between the 9th and 10th thoracic vertebrae. This cut extends approximately 35 cm from the lateral edge of the *longissimus* muscle amongst the ribs, with the vertebrae, connective tissues and the *multifidus dorsi* completely severed. The second cut is made within the connection of the sirloin and the round muscles, severing the *ischium* of the pelvic bone and the intersection between the 4th and the 5th sacral vertebrae including all its surrounding connective tissues. The ischium can be broken as an addition to increase the tension on the hindquarter muscles (Wang *et al,*1994 as cited by Ludwig *et al,* 1997) With sufficient stretching, prominent gaps should appear in the loin and sirloin/round cutting areas when implemented correctly (Sorheim & Hildrum, 2002). Slight shifts in these positions were shown to still cause the positive effects as long as significant gaps appear in the loin and sirloin/round cutting areas (Sørheim *et al,* 2001; Sørheim & Hildrum, 2002). To implement this technology, no new equipment is required and it is adaptable to the current design of existing packing plants. Therefore, the Tendercut process could have immediate benefits on the improving of tenderness scores for certain muscles (Ludwig *et al,* 1997). This method has been legalized in the USA, Canada and Norway (Sorheim & Hildrum, 2002).

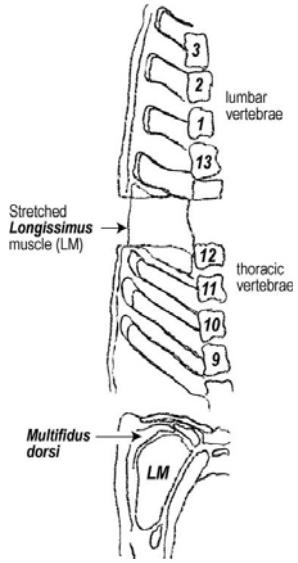


Figure 3.8. Schematic drawing of the Tendercut system with a cut in the 12th/13th vertebrae region of a beef carcass side (Claus, 2002)

3.6.1 Sarcomere length and shear force

Benefits from using Tendercut such as increased tenderness and sarcomere lengths were supported by a number of studies. Increased sarcomere lengths and lower Warner-Bratzler shear force values were found for Tendercut carcasses in the loin and round muscles such as the *Longissimus, Gluteus medius, Vastus lateralis, Rectus femoris, Biceps femoris* and *Vastus medialis,* compared to that from normally Achilles hung carcasses (Wang *et al,* 1994; Claus *et al,* 1997; Claus & Marriott, 1991; Claus *et al,* 1997; Ludwig *et al,* 1997).

Claus *et al* (1997) reported that the most beneficial effect of the Tendercut method was observed in the round muscles *Vastus medialis* and *Rectus femoris*. Variation in the efficiency of TC also exist between the results from Wang *et al* (1994) which revealed higher tenderness scores in round muscles by using TC and those of Beaty *et al* (1999) whose results showed no significant differences for these muscles. In addition, Claus *et al* (1997) found no significant effects for the *Biceps femoris* muscle claiming that it was further away from the affected area and that the muscle probably had more physiologically mature collagen content.

Apart from the *Biceps femoris* muscle, the overall tenderness for Tendercut carcasses showed an increase ($P< 0.05$) in sensory scores of 28.3% for USDA Choice and 18.1% for USDA Select carcasses, compared to the controls whereas WB shear force measurements each scored 17.3% and 10.7% lower than the control for Choice and Select carcasses, respectively (Claus *et al,* 1997).

3.6.2 Rapid chilling

Tendercut, in addition to Tenderstretch, has also proved to have the ability to maintain a satisfactory amount of tenderness while the chilling rate of the carcasses increases (Sorheim & Hildrum, 2002). Consequently, Tendercut had a much greater effect in carcasses that were subjected to cold shortening conditions than those from slow chilling regimes where TC showed very little or no improvement (Dryer *et al,* 1979; Sorheim *et al,* 2001, 2002). Smith *et al* (1971) therefore concluded that by using high temperature chilling regimes up until rigor, no further suspension methods or other tenderising methods were needed.

The Tendercut carcasses rapidly chilled at a constant air temperature of 2°C, had increased sarcomere lengths of 1.73 to 1.94 µm and reduced WB shear forces values from 87.4 to 67.2 N/cm^2 compared to normally hung carcasses (Sorheim *et al,* 2001). Clause *et al* (1997) proved the potential of this method in US Choice and US Select beef carcasses that had been electrically stimulated and rapidly, spray-chilled. Increased sarcomeres lengths of 1.65 µm to 2.41 µm and decreased WB shear force values from 3.95 to 3.23 kg were observed in the LD muscles (Sorheim *et al,* 2001). Beaty *et al* (1999) examined the effects of Tendercut on lightweight heifer carcasses chilled at an air temperature of 4°C. Results showed increases of 1.71 to 1.89 µm for sarcomere lengths and lower WB shear force values from 5.2 to 3.3 kg for the *M. Longissimus thoracis* (Beaty *et al,* 1999). Use of CT improved the sarcomere lengths from 1.68 to 1.79 µm and reduced the WB shear force from 4.6 to 3.1 kg for the *M. longissimus lumborum* (Sorheim *et al,* 2001).

3.6.3 Muscle characteristics

Colour

No significant differences were observed for colour (CIELab values) between the TC and normally hung carcasses (Wang *et al,* 1996). Claus *et al* (1997) however discovered lower a* values in tendercut carcasses for the *M. Longissimus* depicting a slightly less red colour at the point of separation between the 12th and 13th thoracic vertebrae. This was due to the fact that nearly 15 cm of the muscle was directly exposed to the chilled airflow as a result of its surrounding muscles and adipose cover being removed from its surface area.

Ribeye area

Although the ribeye area was not significantly smaller in the Tendercut method, Ludwig *et al* (1997) believed that slight reductions in this area could artificially increase the yield grade in those carcasses near the upper range of a certain yield grade. Contrary to the results from Cotroneo (1992) and Wang *et al* (1996) who saw no significant differences in ribeye area, a reduction in ribeye area of 7.87 cm^2 and 10.84 cm^2 for both the USDA Choice and Select carcasses, respectively, were obtained in the Tendercut treated sides from the study of Claus *et al* (1997). This resulted in a higher USDA yield grade for the Choice carcasses which could also mean a decrease in carcass yield for closely trimmed retail cuts.

3.6.4 Time implemented

Studies were done on the effect that Tendercut has on the carcass when it was implemented at different times post mortem. Cotroneo (1992) and Wang *et al* (1994) treated carcasses 45 minutes post mortem, whereas Wang *et al* (1996) made the cuts 90 minutes post mortem and Claus *et al* (1997) between 35 and 40 minutes post mortem. All of these studies showed higher sensory scores, longer sarcomeres lengths and increased tenderness levels for the *Longissimus dorsi, Rectus femoris* and the *Gluteus medius* despite different endpoint temperatures of 66.5, 70.0 and 76.7°C at the different implementation times. Results only differed between carcasses for the *Biceps femoris* muscle where some studies found increased tenderness and others no significant differences between methods. The differences, however, were not a result of the different times that the method was implemented (Claus *et al,* 1997).

3.6.5 Treatment on location

Ludwig *et al* (1997) divided *Longissimus* muscles into 4 zones where the sarcomere lengths differed significantly for the tendercut carcasses whereas sarcomere lengths remained fairly constant in the control carcasses over all the zones (Figure 3.9). Ludwig *et al* (1997) also revealed a subsequent increase in the tenderness levels over all four segments.

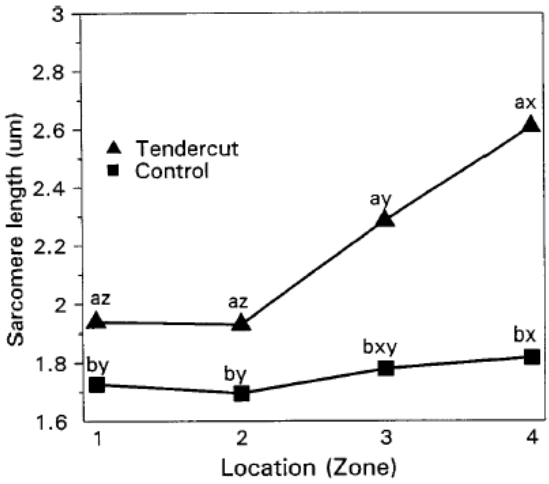


Figure 3.9. Effect of zone on sarcomere lengths by stretching pre-rigor beef. [a,b] Means within the same zone with identical letters are not different (P > 0.05; SE = .078). [x,y]Means within the same pre-rigor treatment with identical letters are not different (P >0.05, SE = .059)

Although no differences for the mechanical measurements of tenderness were found over all 4 zones of the *Longissimus* muscle, a definite increase (P< 0.05) in sensory scores were observed during sensory evaluations (Figure 3.10) (Ludwig *et al,* 1997). Zones 3 and 4 had the highest juiciness scores for the Tendercut carcasses. It was thought that these zones were more affected by the Tendercut method as they were closer to the implement area and thus have longer sarcomere lengths (Table 3.3). In addition these elongated sarcomeres had more intracellular spaces between the filaments in the vicinity of the I-band which improves its ability to hold water and was therefore perceived as being more juicy (Ludwig *et al,* 1997). Additionally, less overlap between actin and myosin filaments exists in stretched sarcomeres, thus also increasing the exposure of hydrophilic regions to connect with water (Claus *et al,* 1997).

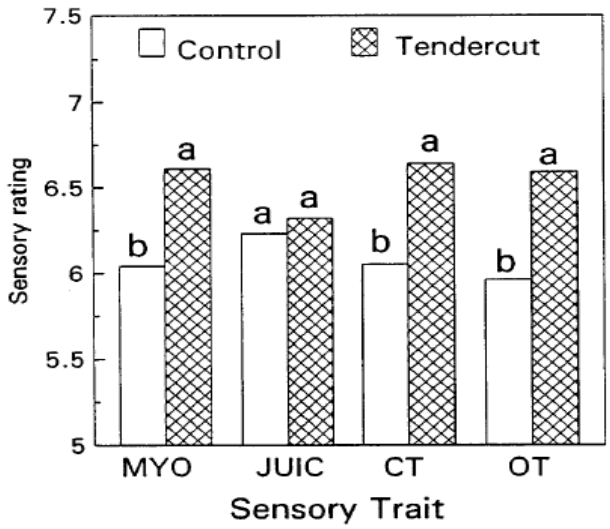


Figure 3.10. Effect of prerigor treatment on sensory panel ratings of beef steaks. a, b Means within an individual sensory trait with unlike letters are different ($P < .05$). Sensory trait: myofibrillar (MYO) and overall tenderness (OT): 1 = extremely tough, 8 = extremely tender; juiciness (JUIC): 1 = extremely dry, 8 = extremely juicy; connective tissue (CT): 1 = abundant, 8 = none. Standard errors: .17 MYO, .053 JUIC, .12 CT, and .16 OT (Ludwig *et al,* 1997)

Table 3.3. Effects of zone on various physical and sensory traits of beef longissimus muscle by treatment (Ludwig *et al,* 1997)

	Zone 1		Zone 2		Zone 3		Zone 4		
Item	Ctrl[a]	TC[a]	Ctrl	TC	Ctrl	TC	Ctrl	TC	SE
Instrumental tenderness									
WB peak force. kg[b]	4.1[c]	3.6[c]	4.0[c]	3.6[c]	4.2[c]	3.7[c]	3.6[c]	3.1[c]	. 26
Sensory trait[e]									
Myofibrillar tenderness[f]	6.6[c]	6.8[c]	5.7[d]	6.3[c]	5.8[d]	6.5[c]	6.0[d]	6.8[c]	.26
Juiciness	6.4[c]	6.2[d]	6.3[c]	6.3[c]	6.1[d]	6.4[c]	6.2[d]	6.4[c]	.20
Connective tissue	6.0[d]	6.8[c]	5.9[d]	6.4[c]	6.1[d]	6.7[c]	6.2[d]	6.8[c]	.28
Overall tenderness[f]	6.2[d]	6.7[c]	5.7[d]	6.4[c]	5.8[d]	6.6[c]	6.1[d]	6.7[c]	.26

[a] Ctrl = control; TC = Tendercut.

[b] WB = Warner-Bratzler shear force.

[c,d] Means within the same row and day with identical superscripts are not different ($P > .05$).

[e] Sensory trait: Myofibrillar and overall tenderness: 1 = extremely tough, 8 = extremely tender; Juiciness: 1 = extremely dry, 8 = extremely juicy; Connective tissue: 1 = abundant, 8 = none.

[f] Treatment x day interaction ($P < .05$)

These results were in contrast to previous studies by Wang *et al* (1996) who discovered no significant differences by using tendercut on the myofibrillar and overall tenderness.

3.6.6 Variance

Adding to the different effects Tendercut has on the different areas across the length of the *Longissimus* muscle, Claus *et al* (1997) and Sørheim *et al* (2001) indicated, through sensory analysis, that the variation between tenderness scores between and within beef *longissimus* muscles also decreased when the tendercut method was implemented (Figure 3.11).

Even though sensory differences occurred in the extent to which each animal (16 Choice cattle) reacted to the TC treatment, the overall variation between the TC treated sides were much lower than between the Control sides, especially in those cattle which were more likely to be tough (Claus *et al,* 1997).

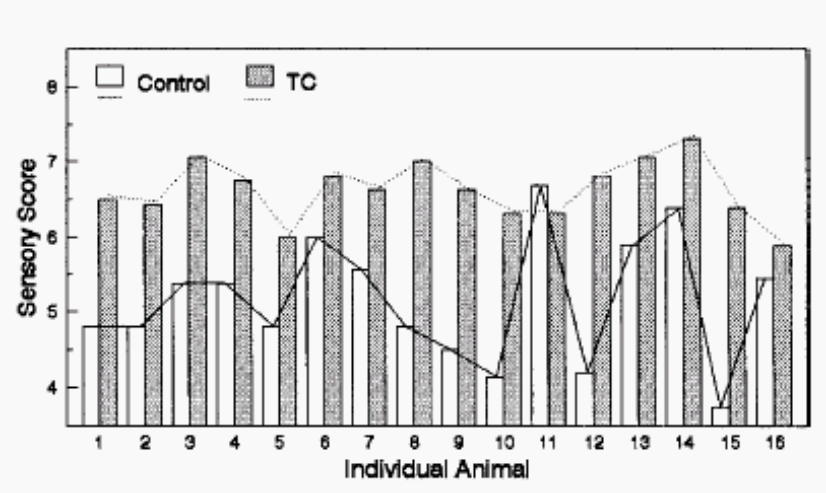


Figure 3.11. Overall tenderness per individual animal for the prerigor carcass muscle stretching treated (TC) and control treated USDA Choice Beef Longissimus muscle steaks (Commercial Testing). Sensory scale for overall tenderness: 1=extremely tough; 8=extremely tender (Claus *et al,* 1997)

3.6.7 Effect of aging on physical and sensory traits

Ludwig *et al,* (1997) tested a treatment x day interaction and discovered that the Tendercut samples were as tender on 3 days of aging as those from the control samples aged for 10 days (Table 3.4). No differences for thawing and cooking loss were found between treatments (Ludwig *et al,* 1997) and collagen solubility remained unaffected (Claus *et al,* 1997).

Table 3.4. Effects of aging on various physical and sensory traits[a] of beef *Longissimus* muscle by treatment (Ludwig *et al,*, 1997)

	Day				
	3		10		
Item	Ctrl [b]	TC [b]	Ctrl	TC	SE
Instrumental tenderness					
Peak force kg	4.4[c]	3.8[c]	3.5[c]	3.2[c]	.30
Sensory trait[e]					
Myofibrillar tenderness[f]	5.6[d]	6.5[c]	6.5[c]	6.8[c]	.17
Juicyness	6.1[d]	6.4[c]	6.3[c]	6.2[c]	.05
Connective tissue	5.7[d]	6.5[c]	6.4[d]	6.8[c]	.12
Overall tenderness[f]	5.6[d]	6.5[c]	6.3[c]	6.7[c]	.16
Thaw and cooking loss					
Thaw loss %	4.1[c]	4.6[c]	5.3[c]	4.1[d]	.21
Cooking loss %	22.4[c]	22.4[c]	21.4[c]	21.7[c]	.15

[a]Traits determined on cooked product include Warner-Bratzler shear and total energy, sensory traits, and cooking loss. [b]Ctrl = control; TC = Tendercut.

[c,d] Means within the same row and day with identical superscripts are not different ($P > .05$).

[e]Sensory trait Myofibrillar and overall tenderness: 1 = extremely tough, 8 = extremely tender;
Juiciness: 1 = extremely dry, 8 = extremely juicy;
Connective tissue: 1 = abundant, 8 = none.

[f]Treatment ´ day interaction ($P < .05$).

Chapter 4

Practical implications of these methods

When using TS, a slight increase in chiller floor space might be necessary on account of the hind leg hanging at a 90° angle, increasing the carcass cubic area. This could be counteracted by altering the height of the rails to reduce the chilling room area and by using faster chilling regimes subsequently increasing the throughput through the chillers without any negative effects from cold induced shortening (Sorheim *et al,* 2001). Compensating for the increased need for floor space when using these methods is that chilling temperatures can be lowered, ensuring a fast throughput without any unfavourable effects on tenderness.

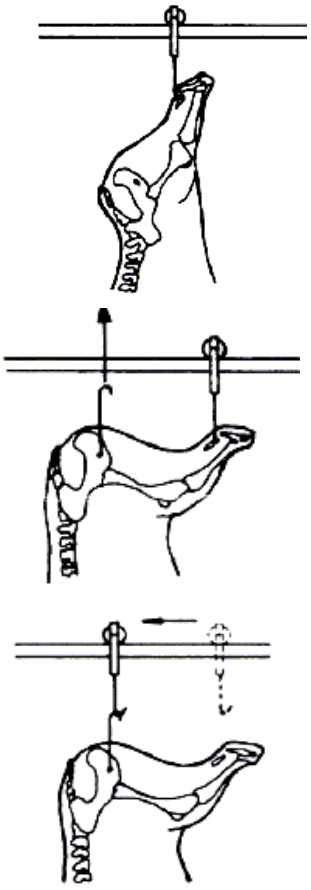
For the TC method the main implication is the fact that it requires more work than the TS method. Well defined criteria need to be given for cutting in the round/sirloin region as it may be difficult finding the exact position to cut without damaging the underlining *M. psoas major*. Although the length of the TC carcass can increase by 15cm to 20cm, no major changes in floor space are required except maybe for the height of the rails (Sorheim *et al,* 2001).

Altered shapes in primal cuts have been found for both methods. LD muscles tend to be longer and slightly smaller in diameter thus producing more, but smaller cuts per muscle (Sorheim *et al,* 2001). According to Tarrant *et al* (1998) the consumer shift towards small pre-packed retail portions might be a beneficiary towards these altered shapes in these specific muscles (cited by Sorheim *et al,* 2001).

Implementing

The Australian meat technology update (1998) (reprinted 2006) supplied detailed descriptions of manual transfer and online transfer procedures for the Tenderstretch method (Figure 4.1 and 4.2 respectfully) (Meat Technology update (1998) newsletter no. 98/2).

Manual transfer method:



Step 1:

Achilles suspension

Step 2:

Hook inserted in eye of aitch bone is raised to take the full weight of the carcass and Achilles tendon suspension removed.

Step 3:

Side is lowered and other end of hook is hooked to conventional slide or roller on the rail.

Figure 4.1. Manual transfers from Achilles tendon to Tenderstretch suspension

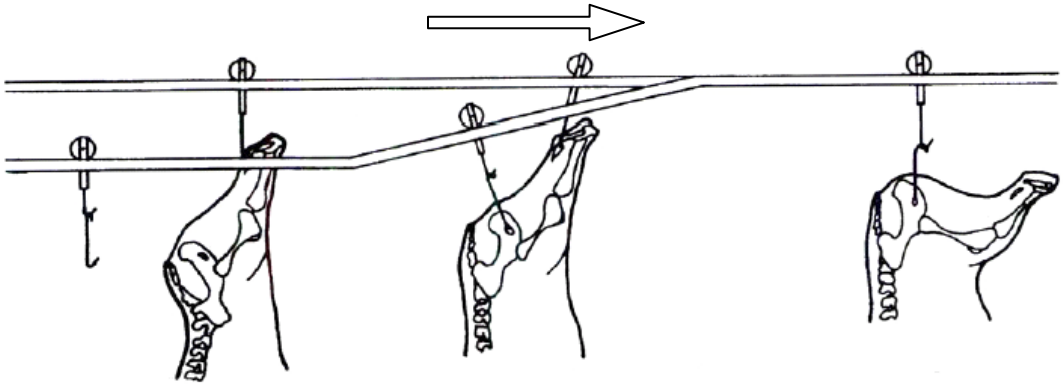


Figure 4.2. Online transfer methods from Achilles tendon suspension to the Tenderstretch suspension

The hind leg, when using Tenderstretch, hanging at 90º towards the spine creates a problem with chiller floor space. Tenderstretch increases the surface area of the carcass and therefore lowers the amount of carcasses per square meter of chiller floor space. In addition to the extra floor space needed to fit in the equal amount of normally hung carcasses, the hanging rails can be lowered thus generating a smaller overall chiller cubic area for more effective chilling.

In contrast to Tenderstretch, the Tendercut method increases the total length of the carcass by as much as 15 to 20 cm. Compensating for the increased chiller space required for these two methods is the possible increased throughput gained by the ability of using higher chilling rates. Tendercut is more labour intensive due to two cuts being made in every carcass, the round/sirloin cut being very specific for satisfactory outcome. A change in shape of some of the primal cuts were observed from using both tendercut and tenderstretch methods (Sorheim & Hildrum, 2002).

Chapter 5

Summary

Low voltage stimulation resulted in large variation in rate of glycolysis and all-round tenderness which could serve as a reason for using restraining or stretching methods such as Tenderstretch and Tendercut (Olsson *et al,* 1994; Sorheim & Hildrum, 2002).

Claus *et al* (1997) showed a decrease in variation for tenderness in the LD muscle by using Tendercut. The same results were found again by Sorheim *et al* (2001) when using TC as well as TS on carcasses exposed to fast chilling regimes. A reduction in sensory tenderness of the LD muscle underlines the importance of these methods to reduce the frequencies of unacceptable tough LDs.

According to Sorheim *et al* (2001), TS were more efficient in increasing the tenderness of the fast chilled LD muscles than those that underwent TC. Since these two methods were conducted in two different experiments, no direct comparisons can be made between these two methods. The MSA found the tendercut method to be less effective than the Tenderstretch method when taking the entire round and loin muscles into account. These results, plus the fact that implementing the tendercut method requires trained, experienced personnel to make the specific cuts, made the Tenderstretch method more likely to be included in the MSA model (Thompson, 2002).

According to the USA national tenderness surveys and registrations from other countries, more research is still needed for the processing, preparation and ageing of the meat (Sorheim & Hildrum, 2002) to alleviate the unacceptable tenderness variations within retail beef cuts from a variety of retail stores (Brooks *et al,* 2000). Pre-slaughter factors such as the number of days the animal is fed a high-energy diet (Tatum *et al,*1980; Dolezal *et al,* 1982; Van Koevering *et al,* 1995), the health status of the animal during its growing and finishing periods (Gardner *et al,* 1999), age at castration (Martinez-Peraza *et al,* 1999), intramuscular injection of animal health products (George *et al,* 1995), temperament or ante mortem stress (Voisinet *et al,* 1997), age (Wulf *et al,*1996a), relative fatness of the animal at slaughter (Dikeman, 1996) (Tatum *et al,* 1999) breed type (Thompson, 2002) and pre-slaughter stress (Tatum *et al,* 1999; Devine, 2001; Thompson, 2002) have repeatedly been proven to have a great influence on the tenderness of the meat.

Even if the mean glycolytic rate coincides with the optimal pH/temperature window, the within lot carcasses' rigor temperature (pH 6.0) still varies between 1.3 to 8.3° which makes it difficult to predict or optimise the subsequent eating quality of the carcass group or lot (Thompson, 2002). The degree of myofibrillar contraction is directly dependable on these two key factors namely the rate of pH decline and

its correlation to the temperature at the onset of rigor mortis (O' Halloran *et al,* 1997; Hannula & Puolanne, 2004).

Since the work of Locker (1960), Marsh and Leet (1966), Herring *et al* (1965a, b; 1967a, b), Davey *et al* (1967) and now Koohmaraie (1996b) showed that the state of contraction of the muscle fibres greatly influenced the tenderness and that the role of connective tissue was demoted to that of background toughness, it is now established that both the fibres and its associated connective tissues contributes to toughness.

This relationship between sarcomere length and tenderness is strongly influenced by the degree of post mortem tenderisation whereas a very low relationship between sarcomere length and tenderness can be expected when the post mortem tenderisation is at a high level as well as the opposite, being a high relationship coinciding with low post mortem tenderisation (Koohmaraie, 1996).

It is concluded that the rigor-induced sarcomere shortening was mainly responsible for meat toughening occurring during the first 24 hours post mortem. The amount of correlation between sarcomere length and tenderness 24 hours post mortem is therefore dependent on the extent of tenderisation occurring during the shortening phase of rigor. Thus the shear force of a muscle at any given time is a direct result of the relationship between the two conflicting agents, sarcomere shortening and tenderising. Therefore, it is either necessary to minimise the toughening phase or to improve or accelerate the tenderisation phase for gaining the maximum level of tenderness (Koohmaraie, 1996).

Numerous researchers found that elongated sarcomere lengths caused a reduction in shear force measurements (Hostetler *et al,* 1970, 1972; Bouton & Harris, 1972; Hostetler *et al,* 1972; Bouton *et al,* 1973; Sorheim & Hildrum, 2002; Thompson, 2002) coinciding with a total decrease in variance between animals (Ahnstrom *et al,* 2005; Thompson *et al,* 2005).

Simmons *et al* (1999) and Sorheim *et al* (2001) confirmed that a moderate amount of stretching from as little as 10 ± 15 %, obtained through using TC or TS, was sufficient in reducing the toughness and therefore concluded that the improvement in tenderness was more a function of the restricting of the muscle from contracting than the extending or stretching of the muscles to a maximum length (Sorheim *et al,* 2001).

The combination of Tenderstretch and aging presented a positive effect on the palatability of striploin, outside round and rump muscles (Bouton *et al,* 1973; O'Halloran *et al,* 1998), having an initial tenderness at 2½ days post slaughter that were equal to the tenderness of non-stretched muscles after 2

weeks of aging (Bouton & Harris, 1972). This could in fact be greatly beneficial for commercial conditions such as reducing the turnaround time of a production plant (O'Halloran *et al,* 1998). Due to the slower aging rates observed in the TS muscles (34 % less than the control at 5 days of aging) the palatability of the meat should converge with the ongoing aging period (Thompson, 2002).

In addition to the lower initial tenderness at the onset of aging, the variance in tenderness between and within the *longissimus* muscle was lower for the Tenderstretch (Sorheim, 2001; Thompson *et al,* 2002; Thompson *et al,* 2005) and tendercut treatments (Claus *et al,* 1997; Sørheim *et al,* 2001) varying in degree of effectiveness between carcasses, having the most effect in the carcasses that were hereditarily tougher. *Semimembranosus* muscles also had lower variance within treatment with a coefficient of 12 % for the Tenderstretch method compared to the 26 % from the normally hung carcasses (Sorheim, 2001).

Although Tenderstretch may help alleviate decreased juiciness from ES carcasses whilst ES decreases the cooking loss found with Tenderstretch carcasses (Bouton *et al,* 1973; Ferguson *et al,* 1999), the effect that hip suspension had on ES or 7 days of aging or with the combination of ES and 7 days of aging on tenderness levels were found to be of no significance (Dransfield *et al,* 1991; Derbyshire *et al,* 2007). A number of studies support the positive effects from using Tenderstretch on meat tenderness of beef, lamb and pork when being rapidly chilled and having the risk of cold shortening (Bouton *et al,* 1973; Hostetler *et al,* 1975; Joseph & Connolly, 1977; Dreyer *et al,* 1979; Sorheim *et al,* 2001; Sorheim & Hildrum, 2002; Thompson, 2002; Thompson *et al,* 2006;). Thompson (2002) found the largest improvement in tenderness for Tenderstretch when carcasses were subjected to rapid chilling regimes where most of the normally hung sides cold shortened. The lack of effect has also been reported during medium chilling rates, which emphasises the importance of the correct processing procedures ensuring the optimal temperatures for rigor mortis (Dreyer *et al,* 1979; Sorheim *et al,* 2001).

Tendercut, in addition to Tenderstretch, has also been proven to have the ability to maintain a satisfactory amount of tenderness while increasing the chilling rate of the carcasses (Sorheim & Hildrum, 2002) and very little or no improvement for those from slow chilling regimes (Dryer *et al,* 1979; Sorheim *et al,* 2001; Sorheim & Hildrum, 2002). Smith *et al,* (1971) therefore made the statement that by using high temperature chilling regimes up until rigor, no further suspension methods or other tenderising methods were needed.

Additionally, the positive effect of Tenderstretch, were only noted when the samples were cooked at 80°C whereas the normally hung carcass samples were more tender than TS sides when cooked between 55 and 60°C (Eikelenboom *et al,* 1998).

Since the TC method was found to be less effective than the TS method for improving the tenderness in the loin and the round muscles, the MSA found it more likely to implement the TS method in their production model (Thompson, 2002). The fact that most of these studies were conducted in two different experiments makes it difficult to make any direct comparisons between these two methods, underlining the importance of implementing both methods in the same environment (Sorheim *et al,* 2001).

Taking all the effects of TS and TC in account, it is still very difficult to assess which treatment to use without implementing both treatments in the same environment for a study and to assess whether it is viable to be used in today's production systems at all.

Firstly, no significant differences were noted when TS and TC were used on the sufficient ES carcasses and when the carcasses entered rigor mortis at the optimal temperature. In addition, the effect of these stretching methods only applies to meat that has been cooked to at least 80°C which is seen as being well done.

Secondly, the fact that certain muscles actually decreased in toughness by using these methods, makes it unclear as to the total economical effect of these methods. It is known that some retailers demand the use of TS method in the United Kingdom. However, for South Africa, being notorious for its biltong production from round muscles and the use of these muscles in processed meats and slow roasts, which requires a slightly tougher structure, the question is asked whether it is necessary to implement these methods in our current production system.

Thirdly, the fact that some beef producers have such a high throughput through the abattoir means that their steaks reach the supermarkets just after 4 to 6 days of aging. If the use of TS and TC can make a positive contribution in tenderness during the first 6 days of aging it can ensure more tender meat in retail. In addition, if an earlier distribution contributes to a higher throughput through the abattoir, this could mean a more economical use of storage chillers. The fact that the use of TS might reduce the amount of chiller space due to a larger carcass area might counteract the former advantages.

These are all questions that will be addressed in the following research chapters.

Chapter 6

Materials and methods

6.1 Pilot study

A pilot study was conducted in order to become familiar with the techniques that were to be utilised in the following trials and to obtain useful information that would aid in the subsequent experimental design.

Animals

Four Bonsmara type cattle from Braams feedlot, designated carcass samples 1 - 4, were slaughtered at Roelcor Abattoir in Malmesbury, Western Cape, South Africa. All four castrated cattle were in lairage for 24 hours before slaughter and had an age classification of A, with carcass weights ranging between 150 - 274 kg (Table 6.1). Carcasses were divided in half and all the left sides were hung by the traditional Achilles suspension method, these serving as the hock suspension method (HS). The right sides from carcasses 1 and 2 were treated using the Tendercut (TC) method and those from carcasses 3 and 4 were treated using the Tenderstretch (TS) technique.

Table 6.1. Carcass weights and classifications from carcasses used in pilot study

Animal	weight	age	Body comp	Fat
1	151	A	3	2
2	203	A	3	0
3	243	A	3	1
4	274	A	3	2

Tenderstretch

Carcass sides were suspended by placing an S-shaped hook in the eye of the aitchbone (*Obturator foramen*) as an alternative to the conventional Achilles tendon suspension position. Carcasses were lifted from the aitchbone by means of a pulley, releasing the hook from the Achilles tendon as soon as the carcass reached the appropriate height. This permitted the hind leg to hang free at a 90° angle towards the vertebrae whilst the carcass was hanging from the aitchbone. This procedure was implemented as the carcasses entered the chiller between 60 - 80 minutes post mortem.

Tendercut

The Tendercut procedure required making cuts in the pre-rigor carcasses whilst suspended using the conventional Achilles tendon method. Two positions were selected where the bone, connective tissue, adipose tissue and some minor muscles were severed to allow the muscles to stretch under the weight of the forequarters.

The first cut was instigated at the routine severing point of the hind and forequarter between the 9^{th} and 10^{th} thoracic vertebrae. This position may differ between various abattoirs. This cut extended *ca.* 35 cm from the lateral edge of the *longissimus* muscle disconnecting the bone, connective tissues, and the *multifidus dorsi* muscle, continuing between the 9^{th} and 10^{th} rib in the direction of the flank. The second cut commenced within the junction between the sirloin and round muscles, severing the *ischium* from the pelvic bone and the intersection between the 4^{th} and the 5^{th} sacral vertebrae including all its surrounding connective tissue. Care was taken not to damage the *Psoas major* muscle on the inside of the carcass.

pH and Temperature

Temperature and pH loggers, set to record measurements every 15 minutes, were inserted in the LD and Round muscles from the right and left sides of all carcasses whilst in chilled storage.

Cubic area measurements

Measurements (length x side x width from inside to outside of carcass) of each carcass side having received different treatments (HS, TC and TS) were taken with a measuring stick to calculate the mean cubic area (Figure 6.1).

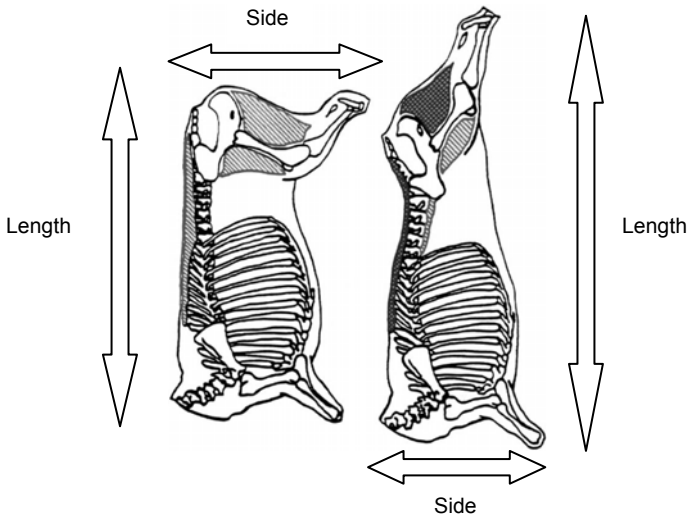


Figure 6.1. Carcass measurements

Laboratory experiments

On the second day following slaughter, the following five muscles were excised at the Roelcor deboning plant, Kraaifontein, Western Cape, South Africa: *Longissimus dorsi, Semimembranosus, Semitendinosus, Gluteus medius* and *Biceps femoris*. Further analyses on these excised muscles were carried out at the Department of Animal Sciences, Stellenbosch University, South Africa.

i) Muscle treatment

On day 2 after slaughter, two steaks (*ca.* 2.5 cm thick) from every muscle were cut perpendicular to the orientation of the muscle fibres, one of which was used for colour evaluation and sarcomere measurements, and the other for cooking loss and shear force measurements. The remainders of the intact muscles were vacuum packed and stored at 4°C until the subsequent day of testing. The same procedures were repeated for days 10 and 21 of aging.

ii) Purge

The weight of every remaining intact muscle, from which steaks were cut, was recorded before vacuum packing on day 2 and there after on removal from vacuum packaging on days 10 and 21 of aging. The purge loss between days 2 and 10, as well as between days 10 and 21, was calculated according to the following formula:

$$\text{Purge loss} = \frac{[\text{Weight before vacuum packing (g)} - \text{Weight after vacuum packing (g)}]}{\text{Weight before vacuum packing (g)}} \times 100$$

iii) Cooking loss

The steaks designated for the cooking loss experiments were cooked in a plastic bag in a water bath at 80 °C to an internal temperature of 70°C following the AMSA cookery guidelines (AMSA, 1978). Cooking loss was calculated according to the following formula:

$$\text{Cooking loss} = \frac{[\text{Raw weight (g)} - \text{Cooked weight (g)}]}{\text{Raw weight (g)}} \times 100$$

iv) Shear force

After the cooked steaks had cooled to room temperature, five 1.27cm diameter cylindrical core samples were cut parallel to the muscle fibre direction of each cooked piece of muscle at randomly chosen sites and an average shear force value (kg/1.27cm) was then calculated (Voisey, 1976) by using Warner Bratzler shear force machine. Care was taken to avoid cutting into visible connective tissue

v) Sarcomere length

Two muscle samples of *ca.* 3 – 4 g were cut from each steak on each sampling date. Each sample was placed in an immersion buffer containing 2.5% (v/v) glutaric dialdehyde (Saarchem, supplied by Merck, Cape Town, South Africa), 0.1M potassium chloride (Saarchem), 0.039 M boric acid (Saarchem) and 5 mM Ethylenediaminetetraacetic acid disodium salt dehydrate (EDTA/ Tritriplex III) (Saarchem) for 24 hours at 4°C. Thereafter, samples were transferred to a storage buffer containing 2.5% glutaric dialdehyde (Saarchem), 0.25 M potassium chloride (Saarchem), 0.29 M boric acid (Saarchem), 5 mM Ethylenediaminetetraacetic acid disodium salt dehydrate (EDTA/ Tritriplex III) (Saarchem) at 4°C until homogenising. Samples were homogenised with a Polytron homogenizer (Lasec, Cape Town, South Africa) at a speed of 27000 min^{-1} for 20 seconds. Homogenised samples were stored at 4°C until microscopic analyses.

For microscopic analyses, three small samples from each homogenised muscle sample were spread out on a glass slide, moistened with storage buffer and covered with a cover slip. Ten images of individual muscle fibres were taken using a digital camera (Nikon, DXM 1200 USA) connected to a light microscope (Nikon, Eclipse E600) using a 40x objective accompanied by the Nikon, ACT-1, USA software program. The Simple PCI (Version 4.0, Compix Inc. USA) image analyses software program was used to measure the sarcomere lengths by counting 3 sets of 10 consecutive sarcomeres from each fibre image. The mean sarcomere length was then calculated for each muscle fibre image (Botha *et al*, 2006).

6.2 Main trial

This trial was conducted at Chalmer Beef Feedlot and Abattoir in Boksberg, Gauteng Province, South Africa.

Experimental design

A total of 32 cattle were divided into four groups of eight each (A1-8, A9-16, A17-24) to be slaughtered on subsequent days over a period of 45 days. All the cattle were of Bonsmara type. Cattle had been castrated at processing entering the feedlot and most of them had an age classification of A (no teeth) at slaughter whereas three cattle had an AB (one or two teeth) classification. Carcass weights (all extra large frame) ranged between 281 kg and 415 kg. All the carcasses had slaughter percentages of 63% to 66% of the live weight (Table 6.2).

The three treatments, Tenderstretch (TS), Tendercut (TC) and Hock suspention (HS) were randomly allocated to each of the 48 sides from group A.

Table 6.2. Carcass weights, age, body composition and fat classifications for the four slaughter groups

Animal	Live weight (kg)	Warm carcass weight (kg)	dressout %	age	body conformation	fat
A1	560.5	367.9	65.64	A	3	3
A2	555	369.2	66.52	A	3	2
A3	574.5	373	64.93	A	3	3
A4	579	366.3	63.26	A	3	3
A5	480.5	306.6	63.81	A	3	3
A6	434	281.7	64.91	A	3	2
A7	522	340.5	65.23	A	3	2
A8	535.5	355	66.29	A	3	2
Average	530.125	345.025	65.08	A	3	2.5
A9	519.5	332.2	63.95	A	3	2
A10	621	415.9	66.97	A	3	4
A11	481.5	304.2	63.18	A	3	2
A12	614	387.5	63.11	A	3	2
A13	561.5	366.3	65.24	A	3	4
A14	530	350.6	66.15	A	3	3
A15	509	330	64.83	AB	3	2
A16	566.5	367	64.78	AB	3	2
Average	550.375	356.7125	64.81	A	3	2.625
A17	527	351.8	66.76	A	3	2
A18	558	364.2	65.27	A	3	2
A19	611	405.8	66.42	A	3	2
A20	526.5	341.3	64.82	A	3	2
A21	559	365.1	65.31	A	3	3
A22	508	346.7	68.25	A	3	2
A23	499	324	64.93	A	3	2
A24	582.5	389	66.78	AB	3	3
Average	546.375	360.9875	66.07	A	3	2.25

Feeding program

On arrival at the feedlot the young animals were fed a dry starter diet for 4 days followed by a different formulated dry starter for another 6 days. For the next 10 days a wet starter containing molasses was fed. The week before and after sorting a high energy production feed was fed after which the animals were placed on their final finisher diet up to the point of slaughter. At this stage the finishing cattle were relocated 17 km to the feedlot situated next to the abattoir where they were finished off for slaughter.

Vaccination, processing and sorting

On day one or two after the weaners arrived, they went through a vaccination program during which they were dipped and inoculated with the necessary antibiotics. At this stage the calves were tagged, sexed and weighed for the first time. On days 5 – 7 the calves were brought back to the crush for further processing where they were weighed, inoculated and the males castrated. At this stage the first hormonal growth promoter (Ralgro) was implanted. Sorting took place at 28 days after arrival at the feedlot where a second implant, Synovex^{+}, was added after the animal was weighed and measured to determine its growth rate.

Slaughter

The eight cattle required per group were selected from the feedlot on the day before slaughter based on conformity and breed. After selection, these cattle were herded *ca.* 400 meters to the lairage area where they were held overnight with sufficient water available until slaughter.

During the slaughter process each animal was stunned with a captive bolt and hoisted up to the rail on its left hind leg. Carcasses were electrically stimulated (ES) with 45V @ 17Hz, (pulse width 5 m/s) for 45 secs immediately after bleeding with an output of 400 milliamps by placing the electrodes in the neck of the carcass. The classification and weight of each carcass was noted. The three treatments, Tenderstretch (TS), Tendercut (TC) and Hock suspension (HS) were randomly allocated to each of the 48 sides from group A.

Tenderstretch (TS)

Carcass sides were suspended by placing an S-shaped hook in the eye of the aitchbone (*obturator foramen*) as an alternative to the normal Achilles tendon position. Carcasses were lifted from the aitchbone by means of a pulley releasing the hook from the Achilles tendon as soon as the carcass reached the appropriate height. This permitted the hind leg to hang free at a 90° angle towards the vertebrae whilst the carcass is hanging form the aitchbone. This procedure was implemented prior to the carcass entering the chiller between 60 and 80 mins after exsanguination.

Tendercut (TC)

The Tendercut procedure required making cuts in the pre-rigor carcasses whilst suspended using the conventional Achilles tendon method. Two positions were selected where the bone, connective tissue, adipose tissue and some minor muscles were severed to allow the muscles (predominately the *Longissimus dorsi*) to stretch under the weight of the forequarters.

The first cut was instigated at the routine severing point of the hind and forequarter between the 9^{th} and 10^{th} thoracic vertebrae. This cut extended *ca.* 35 cm from the lateral edge of the *longissimus* muscle disconnecting the bone, connective tissues, and the *multifidus dorsi* muscle, continuing between the 9^{th} and 10^{th} rib in the direction of the flank. The second cut commenced within the junction between the sirloin and round muscles, severing the *ischium* from the pelvic bone and the intersection between the 4^{th} and the 5^{th} sacral vertebrae including all its surrounding connective tissue. Care was taken not to damage the *Psoas major* muscle on the inside of the carcass.

Achilles Tendon (HS)

These carcasses were suspended in the typical Achilles tendon position and were used as the control for this trial.

Chilling

Carcasses were chilled in drop chillers at an air temperature of -2°C for 16-24 hrs. Only when the internal muscle temperatures were below 7°C, were the carcasses removed from the chiller for further processing.

Cubic area measurements

Measurements of each carcass side (Figure 6.1) having received different treatments (C, TC and TS) were documented with a measuring stick so as to calculate the mean cubic area that that carcass would fill in the chiller.

De-boning

Carcasses implemented with the TS method were re-hung onto their Achilles tendon (the normal Achilles suspension method) prior to being removed from the chiller as the facility did not allow for deboning of TS carcasses. There after the carcasses were split in quarters and moved to the de-boning area for excision of the *Longissimus* and *Gluteus medius* muscles from each side. From every individual muscle, 5 consecutive steaks (2.5 cm thick), , were cut, each being sequentially numbered from the thoracic end 2, 4, 6, 10 and 14 in accordance to the amount of days each steak was to be aged.

Aging

Each steak was weighed and vacuum packed. The vacuum packed steaks were submerged in a 90°C water bath for 1 s to allow “heat shrinkage” of the packaging material; a standard procedure in the meat breaking plant to reduce purge losses. All steaks were kept in a retail chiller at a constant temperature of 3°C. On days 2, 4, 6, 10, and 14 after slaughter, the selected steaks were removed from the chiller for further testing.

Muscle evaluations

i) Purge

The weight of every steak was recorded before vacuum packing and after the removal from the vacuum packaging on days 2, 4, 6, 10 and 14 of aging. The purge loss was calculated according to the following formula:

$$\text{Purge loss} = \frac{[\text{Weight before vacuum packing (g)} - \text{Weight after vacuum packing (g)}]}{\text{Weight before vacuum packing (g)}} \times 100$$

ii) Cooking loss

Thereafter, the steaks were cooked in a plastic bag in a water bath at 80 °C to an internal temperature of 70°C following the AMSA cookery guidelines (AMSA 1978).. Cooked steaks were cooled to room temperature in a cold water bath. Cooking loss was calculated according to the following formula:

$$\text{Cooking loss} = \frac{[\text{Raw weight (g)} - \text{Cooked weight (g)}]}{\text{Raw weight (g)}} \times 100$$

iii) Shear force

After the cooked steaks had cooled to room temperature, 10-20 sample cores (1.27 cm in diameter) were cut perpendicular to the cutting surface of each steak. Every core was sheared using the Warner Blatzer shear force method as described for the pilot study.

iv) Sarcomere length

The same procedures as described in the pilot study were used for the determination of sarcomere lengths.

6.3 Chiller experiment

The objective of this trial was to determine the temperatures for different areas inside the chiller and the effect on the carcass temperature and pH. The effect that the capacity to which the chiller is filled has on the temperature of the chiller was also investigated. To achieve a general conclusion these experiments were divided into 6 groups. The positions of the temperature and pH loggers in groups 2, 4 and 5 are shown in figure 6.2.

Group 1

Ten temperature loggers (EBI-6, Ebro Electronic GmbH & Co. KG. Peringerstraβe 10, D-85055 Ingolstadt, Germany) were positioned at different areas inside a chiller. These were hung from the ceiling halfway between the ceiling and the chiller floor. Loggers were programmed to record measurements every 15 mins. On the first day of the trial, temperatures were measured within the empty chiller. On day two the chiller was filled to its capacity with 120 carcass sides and on day 3 the chiller was emptied again. Temperature measurements were recorded continuously throughout all three days of the trial to assess the effect of warm carcasses on the air temperature and to identify the hotspots within the chiller.

Group 2, 4 and 5

Four carcasses in close proximity to each other were selected for every group in different areas within the chiller. Each of the three group experiments was conducted on a different day. Muscle pH and temperature were measured by using SenTix 41 probes (Germany), connected to portable pH meters 340i (WTW, GmbH & Co. KG, Weilheim, Germany), inserted in the loin and round muscle of every carcass within a group. Loggers were programmed to record measurements every 15 mins. Two to three temperature loggers, measuring the chiller temperature, were placed in the same area as the carcasses tested, to compare with the temperatures from Group 1 to distinguish whether the chiller temperature remained identical over the subsequent experiments.

Group 3

Ten temperature loggers were positioned in the same manner as in group 1 between 120 carcasses over a period of three days (Friday morning to Monday morning). It is standard procedure in this deboning plant to program the chiller to a slower chilling regime over the weekend. No pH or temperature measurements were recorded within the carcasses.

Group 6

Seven carcasses were randomly selected from 120 carcasses throughout different areas within the chiller (figure 6.2), also set to the slower weekend chilling regime. The same temperature and pH loggers were used and they were programmed to record measurements every 15 mins in the round muscles of every carcass for a period of 72 h.

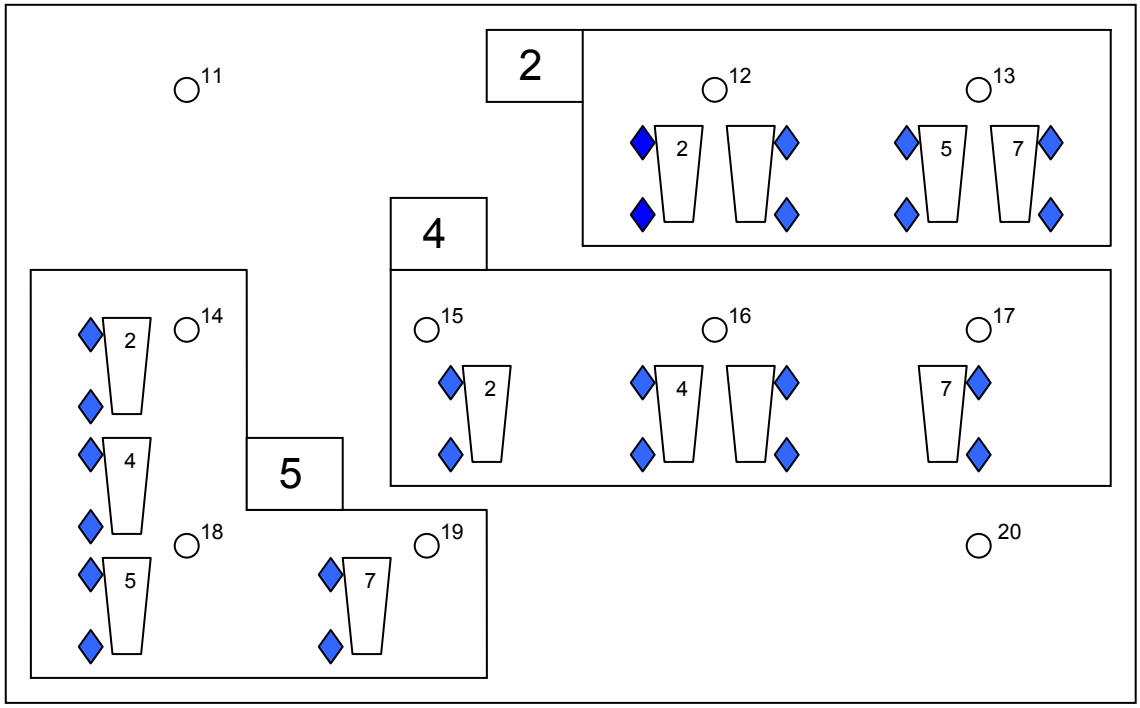


Figure 6.2. Carcass, Temperature and pH logger positions for groups 2, 4 and 5

Group 2, 4, 5.Carcass: Temperature loggers measuring carcass temperature: ◆
Temperature loggers measuring chiller temperature:11 - 20 ○
pH loggers measuring carcass pH: numbers 1 - 7

Statistical analyses

Two types of analyses were done for the pilot study and the main trial.

The experimental design for each pair of carcass sides may be considered as a randomized complete block design with two treatments replicated in 8 block replicates.

Paired t-tests were performed for each pair of treatments and each day separately, on all variables accessed (Snedecor, 1980). Univariate randomized block analysis of variance were performed for each pair of treatments and each day separately, using GLM (General Linear Models) Procedure of SAS statistical software version 9.1 (SAS Institute Inc., Cary, NC, USA). Observations over time were combined in a split-plot analysis of variance with treatment as main-plot factor and day as sub-plot factor (Little, 1972). Shapiro-Wilk test was performed to test normality (Shapiro, 1965). Student's t-least significant difference was calculated at the 5% level to compare treatment means (Ott, 1998). A probability level of 5% was considered significant for all significance tests.

Two similar experiments analyses were also conducted, one for left- and one for right sides of carcasses. For each experiment the experimental design was completely randomized with three treatments each allocated to 8 randomly selected carcasses. Observations were made on day 2, 4, 6, 10 and day 14.

Univariate analysis of variance were performed, for each day separately, on all variables accessed using GLM (General Linear Models) Procedure of SAS statistical software version 9.1 (SAS Institute Inc., Cary, NC, USA). After testing for experiment homogeneity of variance, results of experiments were combined and investigated in one overall analysis of variance (John & Quenouille, 1977). Observations over time were combined in a split-plot analysis of variance with day as sub-plot factor (Little, 1972). Shapiro-Wilk test was performed to test for normality (Shapiro, 1965). Student's t-least significant difference was calculated at the 5% level to compare treatment means (Ott, 1998). A probability level of 5% was considered significant for all significance tests.

Chapter 7

Results and discussion

Four (4) cattle were used for the pilot study, 2 for comparing tender stretch (TS) to hock suspension (HS) and 2 for comparing tender cut (TC) to HS The muscles evaluated were the *Longissimus dorsi* (LD), *Semimembranosus* (SM), *Semitendinosus* (ST), *Gluteus Medius* (GM) and the *Biceps femoris* (BF). These muscles were chosen for their higher market value and their position close to the areas of the treatment's applications. It was speculated that the TS method will have the biggest effect on the GM muscle and the round muscles such as the SM, ST and BF muscles for their position close to the aitch bone where the TS method is implemented. The TC method held the most promise for the LD muscle as it is directly implemented on the LD area although Beaty *et al* (1999) found increased sarcomere length within the SM and ST muscle from using the TC method no significant increase in tenderness were noted. Claus *et al* (1997) found lower shear force values within the GM muscle compared to its controls when using TC method however no improvement in the BF muscle. It was postulated that the location of the BF muscle was too far from the treatment site to be adequately stretched and that the higher amount of collagen could mask the effect of the treatment.

Shear force

Bonferroni (Dunn) t Tests for shear force were conducted for each treatment combination HS – TC, HS – TS and TC – TS per day. Results are shown in Tables 7.1, 7.2 and 7.3 where the means are calculated from subtracting either the treatment TC or TS shear force values from its HS values.

Table 7.1. Paired t-test for shear force differences (kg/1.27 cm) in the LD muscle between the three treatment combinations

Pair= HS -TC Muscle=LD

Day	Difference in Means	Simultaneous 95% Confidence Limits		Significance
2	1.0008	-0.2618	2.2633	ns
10	0.9663	-0.0129	1.9454	ns
21	0.3983	-0.3107	1.1072	ns

Pair= HS-TS Muscle=LD

Day	Difference in Means	Simultaneous 95% Confidence Limits		Significance
2	2.0298	0.7672	3.2923	*
10	0.5587	-0.3528	1.4703	ns
21	0.5999	-0.1349	1.3346	ns

Pair= TC -TS Muscle=LD

Day	Difference in Means	Simultaneous 95% Confidence Limits		Significance
2	1.029	-0.4288	2.4868	ns
10	-0.4075	-1.4801	0.6651	ns
21	0.2016	-0.6394	1.0426	ns

Comparisons significant at the 0.05 level are indicated by *.

Table 7.2. Paired t-test for shear force differences (kg/1.27 cm) in the ST and SM muscles between the three treatment combinations

Pair= HS -TC Muscle=ST

Day	Difference in Means	Simultaneous 95% Confidence Limits		Significance
2	-2.8073	-4.3119	-1.3026	*
10	-0.05625	-0.76225	0.64975	ns
21	0.393	-0.3686	1.1546	ns

Pair= HS -TC Muscle=SM

Day	Difference in Means	Simultaneous 95% Confidence Limits		Significance
2	-0.1783	-0.8208	0.4643	ns
10	-0.265	-0.6344	0.1044	ns
21	0.3043	-0.0469	0.6554	ns

Pair= HS -TS Muscle=ST

Day	Difference in Means	Simultaneous 95% Confidence Limits		Significance
2	0.0727	-1.4319	1.5774	ns
10	-0.04725	-0.75325	0.65875	ns
21	-0.0482	-0.8709	0.7744	ns

Pair= HS -TS Muscle=SM

Day	Difference in Means	Simultaneous 95% Confidence Limits		Significance
2	0.1658	-0.4768	0.8083	ns
10	0.012	-0.3574	0.3814	ns
21	0.1073	-0.2439	0.4584	ns

Pair= TC -TS Muscle=ST

Day	Difference in Means	Simultaneous 95% Confidence Limits		Significance
2	2.88	1.1426	4.6174	*
10	0.009	-0.80622	0.82422	ns
21	-0.4412	-1.3741	0.4916	ns

Comparisons significant at the 0.05 level are indicated by *.

Pair= TC -TS Muscle=SM

Day	Difference in Means	Simultaneous 95% Confidence Limits		Significance
2	0.344	-0.3979	1.0859	ns
10	0.277	-0.1495	0.7035	ns
21	-0.197	-0.6025	0.2085	ns

Comparisons significant at the 0.05 level are indicated by *.

Table 7.3. Paired t-test for shear force differences (kg/1.27 cm) in the GM and BF muscles between the three treatment combinations

Pair= HS -TC Muscle=GM					Pair= HS -TC Muscle=BF				
Day	Difference in Means	Simultaneous 95% Confidence Limits		Significance	Day	Difference in Means	Simultaneous 95% Confidence Limits		Significance
2	0.8137	-0.2768	1.9043	ns	**2**	-0.3998	-1.47	0.6704	ns
10	0.0358	-0.3568	0.4283	ns	**10**	0.1089	-0.963	1.1809	ns
21	-0.2683	-0.7256	0.1891	ns	**21**	0.31	-0.7223	1.3423	ns
Pair= HS -TS Muscle=GM					**Pair= HS -TS Muscle=BF**				
Day	Difference in Means	Simultaneous 95% Confidence Limits		Significance	Day	Difference in Means	Simultaneous 95% Confidence Limits		Significance
2	-0.6328	-2.0407	0.7752	ns	**2**	0.8372	-0.5397	2.2141	ns
10	-0.3462	-0.853	0.1605	ns	**10**	0.5029	-0.8761	1.882	ns
21	-0.0242	-0.6147	0.5662	ns	**21**	0.8655	-0.4672	2.1982	ns
Pair= TC -TS Muscle=GM					**Pair= TC -TS Muscle=BF**				
Day	Difference in Means	Simultaneous 95% Confidence Limits		Significance	Day	Difference in Means	Simultaneous 95% Confidence Limits		Significance
2	-1.4465	-2.9888	0.0958	ns	**2**	1.237	-0.2634	2.7374	ns
10	-0.382	-0.9371	0.1731	ns	**10**	0.394	-1.1088	1.8968	ns
21	0.244	-0.4028	0.8908	ns	**21**	0.5555	-0.9044	2.0154	ns
Comparisons significant at the 0.05 level are indicated by *.					Comparisons significant at the 0.05 level are indicated by *.				

From the pilot study it was noticed that the only significant difference in shear force were noticed for the TS method and only on the second day of aging in the LD muscle (Table 7.1). As the aging of the meat continued, this effect became less prominent. It should be noted that although the HS – TC difference in mean values were all positive, the difference of the individual shear force measurements for both animals were not consistently positive and that some lower negative values were also noticed. This can be seen throughout all 5 muscles and all treatments, where a positive or negative difference in mean values is calculated from both a negative and positive difference in shear force values.

Table 7.2 shows the effects of the three treatments on the ST and SM muscles. Only the ST muscle showed a significant difference in shear force with the TC method. These results showed that the ST muscle was significantly tougher on day 2 of aging when the TC intervention was used. On day 10 a

negative value was noticed whereas day 21 was the only day that the ST muscle undergoing the TC method was slightly more tender than that of the HS method.

The TC also resulted in tougher meat when compared to the TS intervention. For the SM muscle, no significant differences were found between treatments.

The effect of the treatments on the GM and BF muscles are shown in Table 7.3. None of the interventions changed the tenderness of either muscle significantly.

From the five muscles used in this pilot study, only the LD and the GM muscles were chosen to be used in the main study. The LD muscle showed the most promise in conveying an effect due to the treatments and the GM muscle was chosen for its high retail value as a good quality steak. During discussions with Chalmer beef it was decided not to use the ST, SM and the BF muscles as these muscles are more likely to be applied either to the biltong or the processed meat industry in South Africa. As mentioned in literature, various studies showed controversial results for the ST muscles, where even longer sarcomeres, presented tougher meat during shear force measurements due to stretching. This was most probably because of the higher quantity of collagen fibres per cm^2 that has to be cut during WBSF measurements when this muscle is stretched. (Beaty *et al*, 1999)

Purge and cooking loss will be discussed in more depth in the main trial since no differences were noticed in the pilot study.

Chapter 8

Main study

Experimental design

The effects of three types of carcass treatments (HS, TC, TS) were investigated. Each carcass was divided into 2 sides, thus treatments were applied in pairs at random to the left and right sides of a carcass. Each of the three pairs of treatments (HS-TC, HS-TS, and TC-TS) was replicated on 8 carcasses. After 14 days of aging very little increase in tenderness is noticeable and the rate of increase declines strongly after 6 days of aging. Therefore, the observations made on day 2, 4, 6, 10 and 14 post mortem were the optimum amount of days to determine the rate of tenderising. The experimental design for each pair may be considered as a randomized complete block design with two treatments replicated in 8 block replicates.

Paired t-tests were performed for each pair of treatments and each day separately, on all variables accessed (Snedecor, 1980). Univariate randomized block analysis of variance was also performed for each pair of treatments and each day separately (results in the same p-values for the treatment effect as the paired t-test), using GLM (General Linear Models) Procedure of SAS statistical software version 9.1 (SAS Institute Inc., Cary, NC, USA). Observations over time were also combined in a split-plot analysis of variance with treatment as main-plot factor and day as sub-plot factor (Little, 1972). The Shapiro-Wilk test was performed to test for normality (Shapiro, 1965). Student's t-least significant difference was calculated at the 5% level to compare treatment means (Ott, 1998). A probability level of 5% was considered significant for all significance tests.

Shear force

To determine whether a treatment gives more tender meat than the control, the 24 animals were divided into groups of 8 animals each. Each group had a different combination of treatments between its sides, namely HS – TC, HS – TS and TC – TS. Table 4 gives the results of the pair-wise t-Test comparisons between treatments for the GM and the LD muscle. Within the HS –TS group only 7 animals were tested since one of the carcasses' aitchbones were broken when the hook for the hip suspension was applied.

The differences between the treatments HS – TC and HS – TS were determined by subtracting the TC or TS shear force value from their HS sides. For the group TS – TC the difference were obtained by subtracting TS from its TC values. In these Paired t-tests, on the three treatment groups, very few differences were revealed (Table 8.1).

Table 8.1. Paired t-test on shear force for the combinations of HS –TC, HS – TS and TC – TS over 14 days of aging

Shear force (kg/1.27 cm)									
Pair=HS-TC Muscle=GM									
		Difference in	negative		positive				
Day	N	Mean	amount	average	amount	average	Std Error	t Value	Pr > |t|
2	8	-0.2274	6	-0.35917	2	0.168	0.1417	-1.6	0.1527
4	8	0.2095	3	-0.08967	5	0.389	0.1055	1.99	0.0874
6	8	0.0654	4	-0.1445	4	0.27525	0.0935	0.7	0.5068
10	8	-0.0093	4	-0.1845	4	0.166	0.081	-0.11	0.9123
14	8	0.0469	3	-0.189	5	0.1884	0.09	0.52	0.6186

Pair= HS-TC Muscle=LD									
		Difference in	negative		positive				
Day	N	Mean	amount	average	amount	average	Std Error	t Value	Pr > |t|
2	8	0.2855	1	-0.418	7	0.386	0.1489	1.92	0.0966
4	8	0.3205	1	-0.021	7	0.369286	0.0963	3.33	0.0126
6	8	0.1055	4	-0.1735	4	0.3845	0.1321	0.8	0.4507
10	8	0.1275	1	-0.434	7	0.207714	0.0924	1.38	0.2103
14	8	-0.0394	5	-0.205	3	0.236667	0.1128	-0.35	0.7372

Pair=HS-TS Muscle=GM									
Day	N	Difference in Mean	Negative amount	Negative average	positive amount	positive average	Std Error	t Value	Pr > |t|
2	7	0.0317	3	-0.33133	4	0.304	0.1496	0.21	0.8392
4	7	0.0721	4	-0.2795	3	0.402333	0.1691	0.43	0.6846
6	7	0.0011	4	-0.3375	3	0.452667	0.1673	0.01	0.9948
10	7	-0.0807	4	-0.36125	3	0.293333	0.1497	-0.54	0.6091
14	7	0.0784	2	-0.1605	5	0.174	0.0717	1.09	0.3158

Pair= HS-TS Muscle=LD									
Day	N	Difference in Mean	negative amount	negative average	positive amount	positive average	Std Error	t Value	Pr > |t|
2	7	0.2524	2	-0.1495	5	0.4132	0.145	1.74	0.1324
4	7	0.1276	4	-0.0495	3	0.363667	0.0905	1.41	0.2084
6	7	0.2669	2	-0.0325	5	0.3866	0.0929	2.87	0.0283
10	7	0.1681	2	-0.0605	5	0.2596	0.0676	2.49	0.0474
14	7	0.1367	1	-0.153	6	0.185	0.0872	1.57	0.1679

Pair=TC-TS Muscle=GM									
Day	N	Difference in Mean	negative amount	negative average	positive amount	positive average	Std Error	t Value	Pr > |t|
2	8	-0.1091	2	-0.22	6	0.218833	0.0985	-1.11	0.3045
4	8	-0.0611	3	-0.36867	5	0.319	0.1383	-0.44	0.6719
6	8	0.028	5	-0.1334	3	0.147667	0.0722	0.39	0.7096
10	8	0.2353	5	-0.4842	3	0.179667	0.1317	1.79	0.1172
14	8	0.0989	5	-0.403	3	0.408	0.182	0.54	0.6038

Pair=TC-TS Muscle=LD									
Day	N	Difference in Mean	negative amount	negative average	positive amount	positive average	Std Error	t Value	Pr > |t|
2	8	-0.0825	4	-0.17675	4	0.34175	0.1005	-0.82	0.4387
4	8	0.0349	4	-0.25425	4	0.1845	0.1085	0.32	0.7573
6	8	-0.0898	3	-0.25867	5	0.2988	0.1106	-0.81	0.4439
10	8	-0.076	4	-0.18375	4	0.33575	0.1234	-0.62	0.5575
14	8	-0.0716	3	-0.155	5	0.2076	0.0935	-0.77	0.4688

From Table 8.1 it is shown that the TC treatment had no significant effects on the GM muscle.

Although the TC method gave lower shear force values in the GM muscle on days 4, 6 and 14, it was slightly tougher on days 2 and 10. These differences were all less than 0.2274 kg/1.27 cm ∅ in shear force. A similar effect was noticed in the LD muscles subjected to the TC method. No significant differences were obtained between treatments except on day 4 where the highest difference of 0.3205 kg/ 1.27cm ∅ was measured. These mean shear force differences were calculated from the mean shear force values from 8 carcasses of the same treatment combination. From the table it can be seen that the differences between the means for the 8 animals were not consistently negative or positive. This means that the treatment did not show an increase in tenderness for all the animals which means that the effect of these treatments are inconsistent. The reason for the positive and negative values is therefore a result of large positive or negative differences within the same carcass between two treatments. For example, the steaks evaluated on days 6 and 10 from the TC method had 4 carcasses showing an increase in tenderness from using this method and 4 carcasses showing a decrease in tenderness over the HS method. This can be seen throughout all the treatment combinations and on all different days of aging. The fact that the overall difference between mean values showed an increase in tenderness does however not mean that the treatment had an increase in tenderness for all carcasses, which renders these treatments inconclusive.

For the 8 animals where the 2 treatments TS and HS were paired for each animal on opposite sides, no significant improvement in tenderness were noticed when using TS method on the GM muscle. Although significantly smaller shear force measurements were noted for the TS method in the LD muscles, these differences were only illustrated on days 6 and 10 of aging. From these significantly lower shear force values, 2 of the 7 carcasses had a decrease in tenderness from using the TS method (Table 8.1). The method was therefore inconsistent. Differences in the LD muscle between TS and HS were all less than 0.0807 kg / 1.27 cm ∅ with a maximum of 0.2669 kg /1.27 cm.

To determine which of the 2 methods TC or TS had the most tenderising effect, the two treatments TC and TS were compared on each side per animal. This was done to try and eliminate all the external and internal variances between animals. These results were inconclusive. In the GM muscle TS caused slightly less tender meat on days 2 and 4 and more tender than TC on days 6, 10 and 14, however, these differences were never significant and not consistently more tender for all 8 animals. Form the mean difference between the treatments the LD muscle was affected more by the TC method than TS on days 2, 6, 10 and 14 where the meat from these methods were slightly more tender although still not significant and also not consistently more tender for all 8 animals. Mean differences between

these two methods in the GM muscle were 0.2353 kg/ 1.27 cm ∅ and lower whereas the differences in the LD muscle were no larger than 0.0898 kg/ 1.27 cm.

ANOVAs per pair of treatments

Two similar analyses were conducted, using the same results, one for the left sides and one for right sides of the carcasses. For each experiment the experimental design was completely random with three treatments each allocated to 8 randomly selected carcasses. Observations were made on day 2, 4, 6, 10 and day 14.

Univariate analysis of variance was performed, for each day separately, on all variables accessed using GLM (General Linear Models) procedure of SAS statistical software version 9.1 (SAS Institute Inc., Cary, NC, USA). After testing for experiment homogeneity of variance, results of experiments were combined and investigated in one overall analysis of variance (John & Quenouille, 1977). Observations over time were also combined in a split-plot analysis of variance with day as sub-plot factor (Little, 1972). The Shapiro-Wilk test was performed to test for normality (Shapiro, 1965). Student's t-least significant difference was calculated at the 5% level to compare treatment means (Ott, 1998). A probability level of 5% will be considered significant for all significance tests.

From the ANOVA (Table 8.2), a significant difference was found between the animals ($P< 0.0001$) which prove the variance of tenderness that exists between different animals even within the same species. The ANOVA also indicated an effect of the days of aging on meat, where a significant decrease in shear force over time was illustrated. No significant differences were noted between treatments.

Figure 8.1 gives the mean shear force values for treatments TC and HS for each day of aging. It shows that the shear force only significantly lowered after 14 days of aging and never between treatments.

Figure 8.2 indicates the mean difference between the treatments when the Shear force values of TC were subtracted from the HS value at each day of aging (from the paired t-test). Note that the difference, either positive or negative, becomes smaller towards 14 days of aging indicating that the variation between carcasses is becoming less, a result that will lead to a more consistent quality product because of aging. These differences however were not consistently better for all treatments which again make the effects of these treatments inconsistent. The standard deviations in figures 8.1 and 8.2 show how the values for the 8 carcasses overlap between treatments. This again proves the inconsistently of these methods.

Table 8.2. Anova on shear force for Animal, Treatment, Days of aging and the effect of treatment per day

Muscle=**GM** Pair=HS - TC

The GLM Procedure

Dependent Variable: ShearForce

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	23	10.95474	0.4762931	4.12	<.0001
Error	56	6.468088	0.11550156		
Corrected Total	79	17.42283			

R-Square	Coeff Var	Root MSE	ShearForce Mean
0.628758	9.084639	0.339855	3.740988

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GrAnAgeFat	7	5.933447	0.84763533	29.46	0.0001
Treatment	1	0.005797	0.00579701	0.2	0.6671
Error (a)	7	0.201385	0.02876936	0.25	0.9704
Day	4	4.411323	1.10283079	9.55	<.0001
TrtxDay	4	0.402788	0.10069711	0.87	0.4867

Gr = group; An = animal; Age = the age of the animal per classification; Fat = Fatness level of the animal

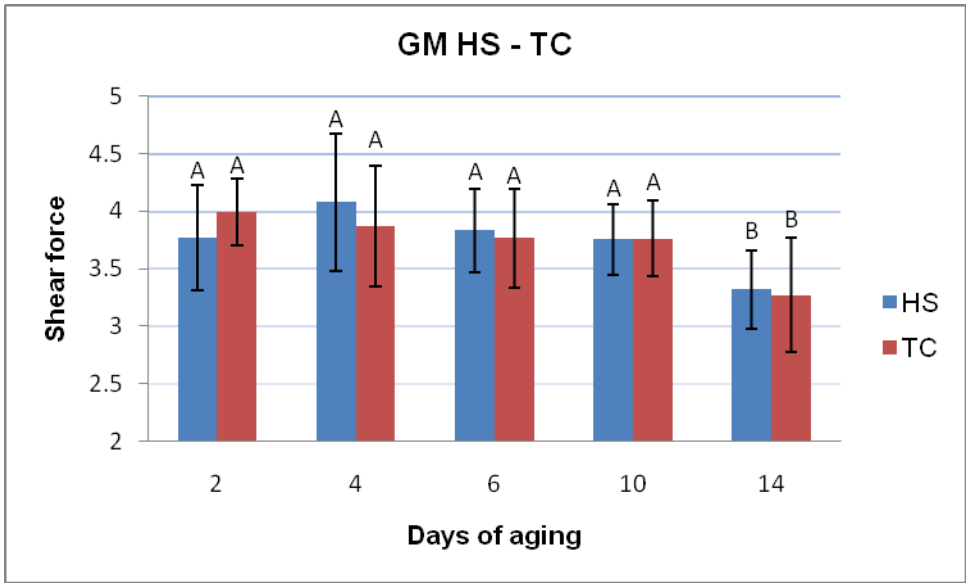


Figure 8.1. The shear force values of HS and TC treatments in the GM muscle over 14 days of aging with standard deviation

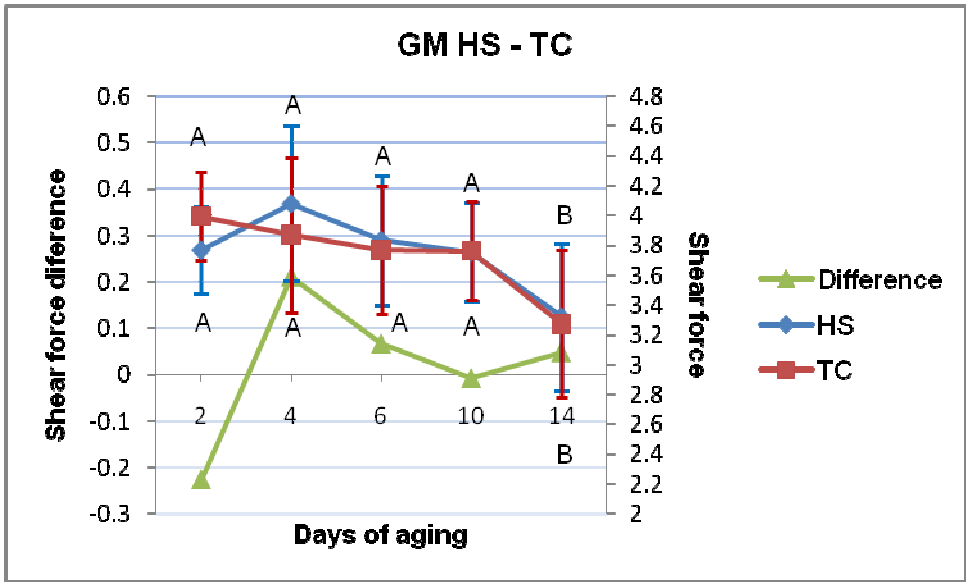


Figure 8.2. The difference in shear force between HS and TC treatments in the GM muscle over 14 days of aging with mean shear force standard deviation

From the ANOVAs on the LD muscle subjected to the TC and HS method (Table 8.3), a significant difference for shear force was found between animals. Differences between the treatments were however not significant and not consistent. Aging of the meat definitely decreased the shear force values in the LD muscle over time.

Figure 8.3 displays the mean shear force values for LD muscles with treatments TC and HS for each day of aging. Although the TC treatment was significantly different on days 2 and 4, the treatment had a less noticeable effect on days 6, 10 and 14. The overall treatment effect was thus not significant (P = 0.0825). Although the standard deviations for the TC treatment were smaller on days 2, 4, 6, and 14, the overlap between the two treatments were still apparent. This shows the inconsistency in effect from using the TC method. The tenderizing effect of aging is however still evident.

Figure 8.4 portrays how the difference in shear force values between HS and TC (from paired t-test) becomes smaller as aging of the meat commences and how the standard deviation overlaps between treatments.

Table 8.3. The Anova for shear force per animal, Treatment, Day and treatment by day

Muscle=LD Pair= HS –TC

The GLM Procedure

Dependent Variable: ShearForce

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	23	14.50558	0.630677	15.08	<.0001

	R-Square	Coeff Var	Root MSE	ShearForce Mean
	0.86102	5.163657	0.204476	3.959913

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GrAnAgeFat	7	4.970067	0.71001	5.69	0.0177
Treatment	1	0.51152	0.51152	4.1	0.0825
Error (a)	7	0.873402	0.124772	2.98	0.0099
Day	4	7.809442	1.952361	46.7	<.0001
TrtxDay	4	0.341149	0.085287	2.04	0.1012
Error	56	2.341391	0.041811		
Corrected Total	79	16.84697			

Gr = group; An = animal; Age = the age of the animal per classification; Fat = Fatness level of the animal

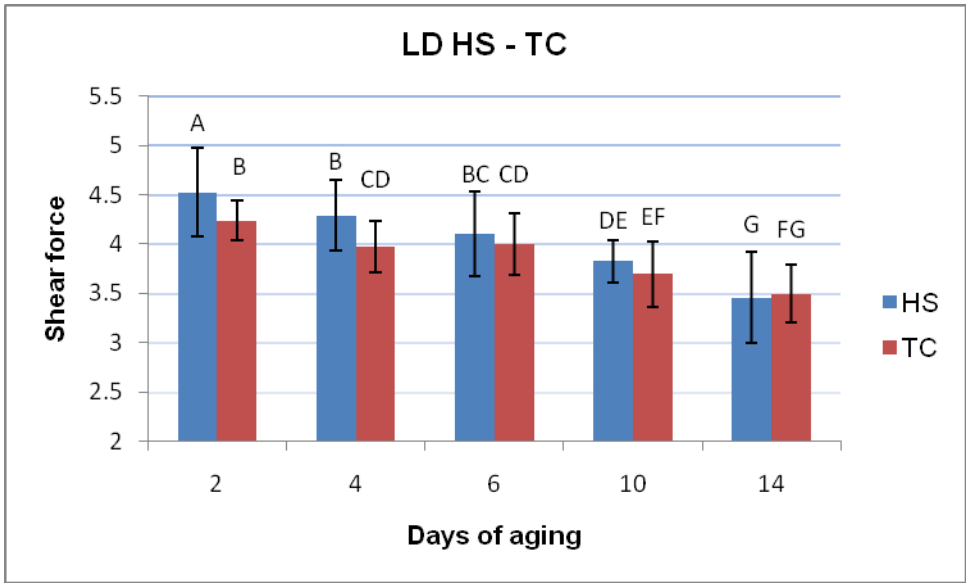


Figure 8.3. The shear force values of TC and HS treatments in the LD muscle over 14 days of aging with standard deviation

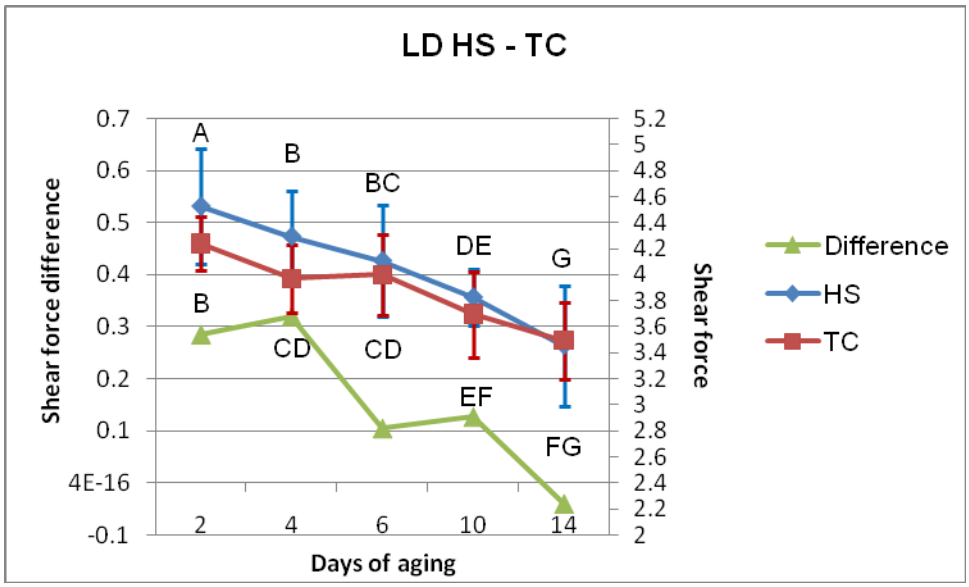


Figure 8.4. The difference in shear force between TC and HS treatments in the LD muscle over 14 days of aging and standard deviation for shear force means

For the group given the treatment combination of TS and HS (Table 8.4) no significant differences were found between animals for shear force of GM. The GM muscle differences were thus more uniform than the previous group. Treatment TS had no effect on the GM muscle. The aging effect however was again significant.

As mentioned, figure 8.5 shows that there were no noteworthy differences between the treatments and that meat aged for 14 days were definitely more tender. It is also evident that the standard deviation of the TS treatment is mostly larger than that from HS, which suggest that these treatments had no definite positive or negative effect on the tenderness of the meat.

Figure 8.6 illustrate the random effect for the treatment TS over HS, giving a positive difference on days 2 and 14 and a negative difference on days 4 and 10. Day 6 showed very little difference. The overlap between treatment standard deviation is also obvious.

Table 8.4. The Anova for Animal, treatment, days of aging and treatment by day

Muscle=GM Pair=HS-TS

The GLM Procedure

Dependent Variable: ShearForce

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	21	4.9087	0.2337	1.81	0.0468

R-Square	Coeff Var	Root MSE	ShearForce Mean
0.446472	9.839432	0.359838	3.657101

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GrAnAgeFat	6	1.6117	0.2686	1.68	0.2715
Treatment	1	0.0099	0.0099	0.06	0.8119
Error (a)	6	0.9576	0.1596	1.23	0.3069
Day	4	2.2155	0.5539	4.28	0.0049
TrtxDay	4	0.1140	0.0285	0.22	0.9259
Error	47	6.0857	0.1295		
Corrected Total	68	10.9944			

Gr = group; An = animal; Age = the age of the animal per classification; Fat = Fatness level of the animal

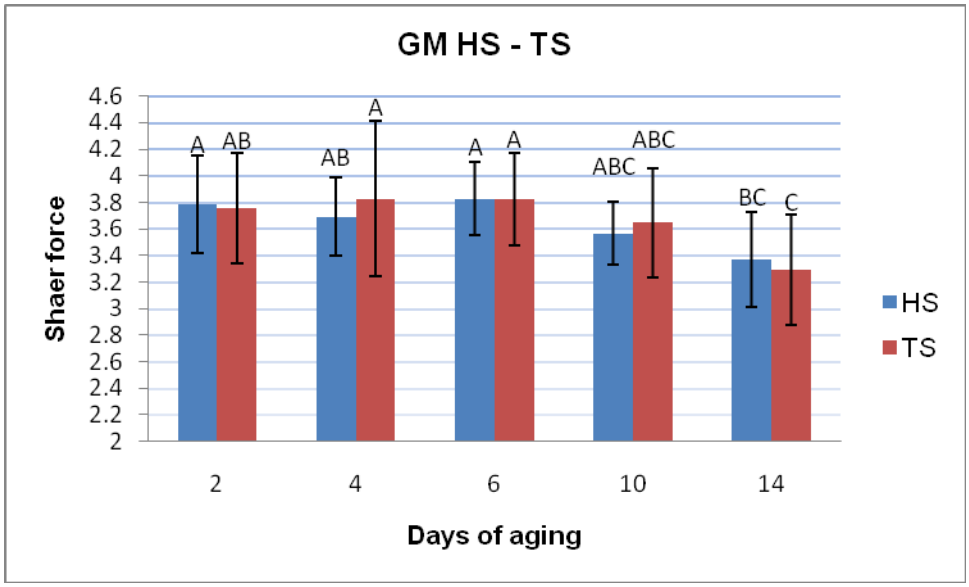


Figure 8.5. The shear force values of TS and HS treatments in the GM muscle over 14 days of aging with standard deviation

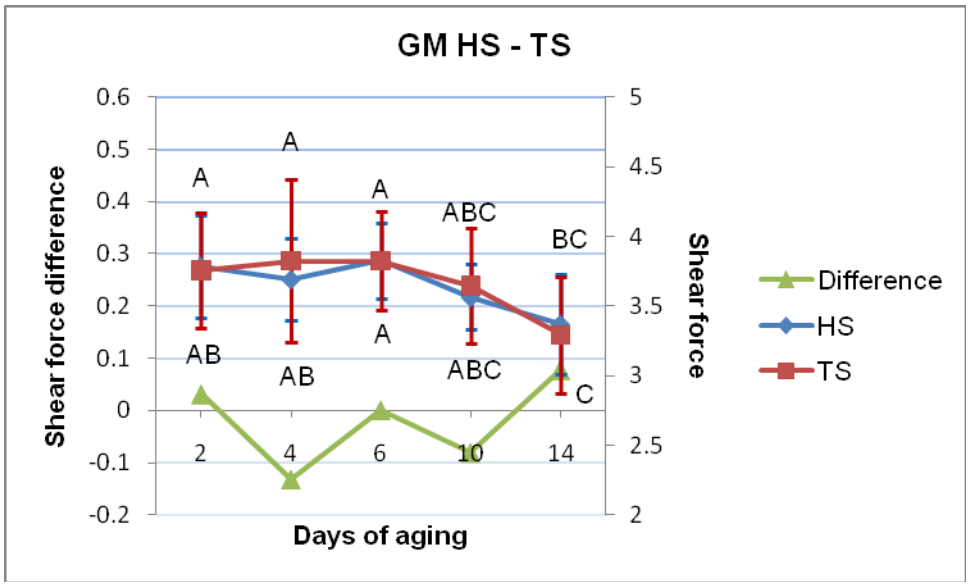


Figure 8.6. The difference in shear force between TS and HS treatments in the GM muscle over 14 days of aging and standard deviation for shear force means

The mean shear force values between the LD muscles for the HS – TS combination differed between animals. Although a significant difference between treatments is shown, this was only noticed on days 2 and 6. This differs from the paired–t Test which gave significant differences on days 6 and 10. Again a difference (P <.0001) is found for the aging effect on meat (Table 8.5).

Figure 8.7 illustrates the positive effect that TS had on days 2 and 6 and how aging significantly lowers the shear force over time. The standard deviations also shows that no treatment produced a increase in tenderness for each animal.

Figure 8.8 shows how the two treatments had a more similar shear force value during the last two days of aging as the difference between the two treatments (from pair t-test) becomes smaller as aging commences except for day 4 where the values are very much the same. Although the HS treatments gave the highest upper values for its standard deviations for all the days of aging, this could be due to chance as some of the TS standard deviations were larger than its opposite HS.

Table 8.5. The Anova for Animal, treatment, days of aging and treatment per day

Muscle=LD Pair=HS - TS

The GLM Procedure

Dependent Variable: ShearForce

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	21	11.06713063	0.52700622	10.14	<.0001

R-Square	Coeff Var	Root MSE	ShearForce Mean
0.81608	5.725775	0.227953	3.981171

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GrAnAgeFat	6	2.43883234	0.40647206	5.26	0.0317
Treatment	1	0.63403206	0.63403206	8.2	0.0287
Error (a)	6	0.46403634	0.07733939	1.49	0.2023
Day	4	7.47066623	1.86766656	35.94	<.0001
TrtxDay	4	0.05956366	0.01489091	0.29	0.8853
Error	48	2.49420131	0.05196253		
Corrected Total	69	13.56133194			

Gr = group; An = animal; Age = the age of the animal per classification; Fat = Fatness level of the animal

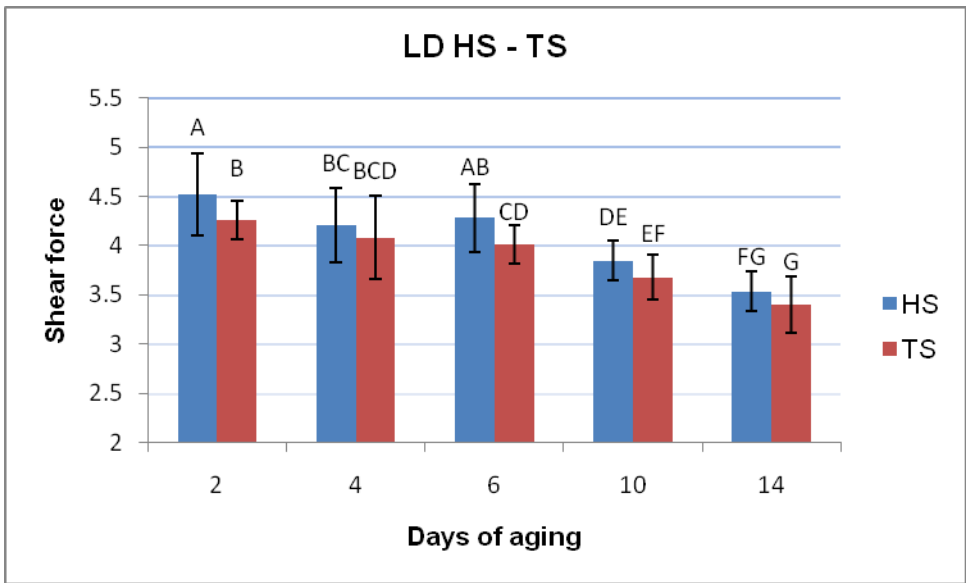


Figure 8.7. The shear force values of TS and HS treatments in the LD muscle over 14 days of aging with standard deviation

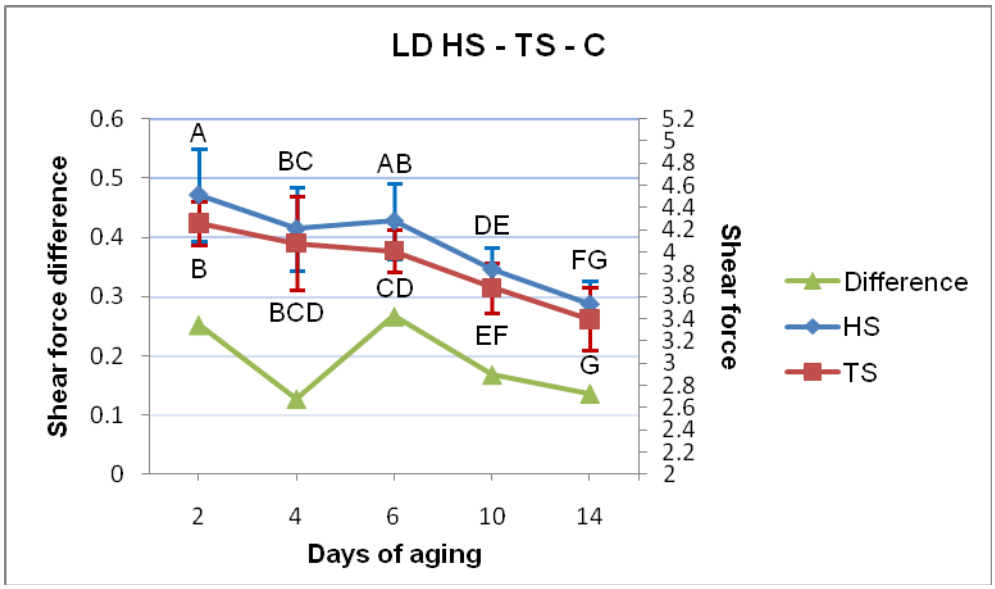


Figure 8.8. The difference in shear force between TS and HS treatments in the LD muscle over 14 days of aging and standard deviation for shear force means

Table 8.6 only gives significant differences for shear force between animals and days of aging. Treatment had no major effect on the GM muscle. Aging again made a significant difference in tenderness as the each day of aging commences.

Figure 8.9 shows that the meat is only significantly more tender after 14 days of aging. The standard deviation shows the overlap of shear force values for each treatment on each day.

Figure 8.10 starts out to show that the differences between these two treatments were small. Although a positive difference of 0.23525 kg / 1.27 cm shear force was found on day 10. This was not significant and on day 14 the difference lowers again below 0.1kg/1.27 cm. The standard deviations again portrays that differences between these 2 methods is very little and that the effects from these methods are very inconclusive.

Table 8.6. The Anova on the group TC – TS for animal, treatment, day and treatment by day

Muscle=GM Pair=TC - TS

The GLM Procedure

Dependent Variable: ShearForce

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	23	8.490553	0.369154	3.15	0.0002

R-Square	Coeff Var	Root MSE	ShearForce Mean
0.564135	9.444857	0.342262	3.623788

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GrAnAgeFat	7	4.003385	0.571912	6.26	0.0136
Treatment	1	0.029453	0.029453	0.32	0.5879
Error (a)	7	0.639322	0.091332		
Day	4	3.521657	0.880414	7.52	<.0001
TrtxDay	4	0.296737	0.074184	0.63	0.6408
Error	56	6.560006	0.117143		
Corrected Total	79	15.05056			

Gr = group; An = animal; Age = the age of the animal per classification; Fat = Fatness level of the animal

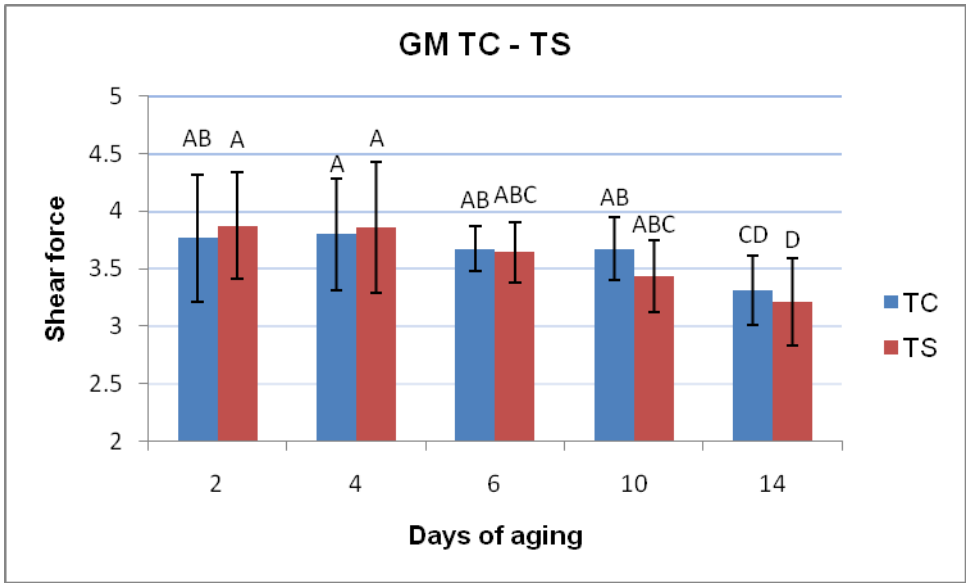


Figure 8.9. The shear force values of TS and TC treatments in the LD muscle over 14 days of aging with standard deviation

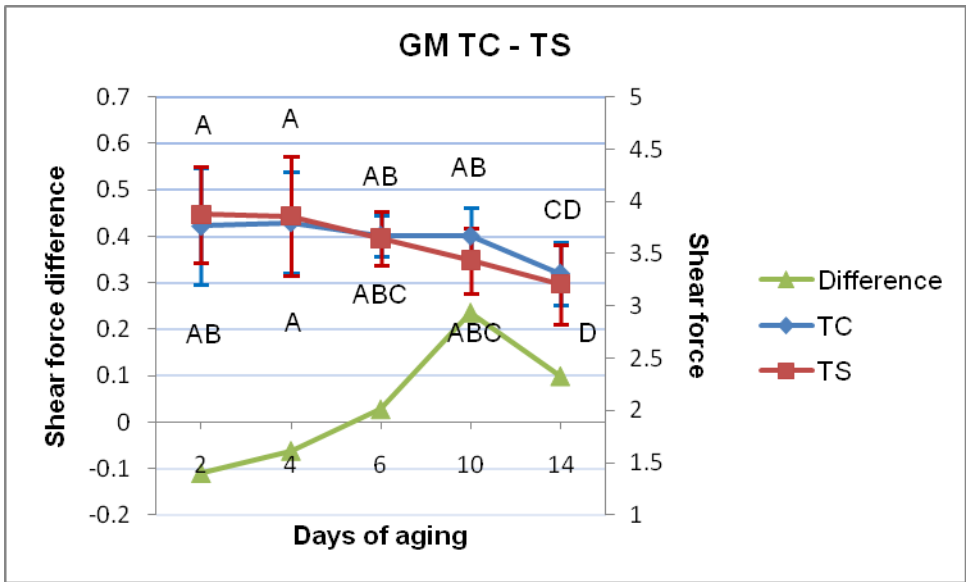


Figure 8.10. The difference in shear force between TS and TC treatments in the GM muscle over 14 days of aging and standard deviation for shear force means

Shear force values from the LD muscles from the group TC – TS were found to be only significant between animals and for days of aging (Table 8.7).

Figure 8.11 portrays the effect of aging on meat tenderness and the insignificant differences between the treatments TC and TS for each day. It also reveals how the standard deviations differ between treatments and within treatments over the days of aging. A higher standard deviation did not mean a higher shear force value or the opposite.

Figure 8.12 shows the small differences in shear force between these two methods and that the TC method produced somewhat tougher meat on day 4 whereas the TS method was less tender than TC on days 6 to 14. The standard deviations show the overlap between treatment values after each day of aging and how the effects of these treatments can be due to chance.

Table 8.7. The Anova on the group TC – TS for animal, treatment, day and treatment by day

Muscle=LD Pair=TS-TC

The GLM Procedure

Dependent Variable: ShearForce

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	23	15.20986	0.661298	13.58	<.0001

R-Square	Coeff Var	Root MSE	ShearForce Mean
0.847924	5.69485	0.22071	3.8756

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GrAnAgeFat	7	5.83006	0.832866	13.86	0.0013
Treatment	1	0.06498	0.06498	1.08	0.333
Error (a)	7	0.420773	0.06011	1.23	0.3
Day	4	8.851093	2.212773	45.42	<.0001
TrtxDay	4	0.042955	0.010739	0.22	0.9259
Error	56	2.727912	0.048713		
Corrected Total	79	17.93777			

Gr = group; An = animal; Age = the age of the animal per classification; Fat = Fatness level of the animal

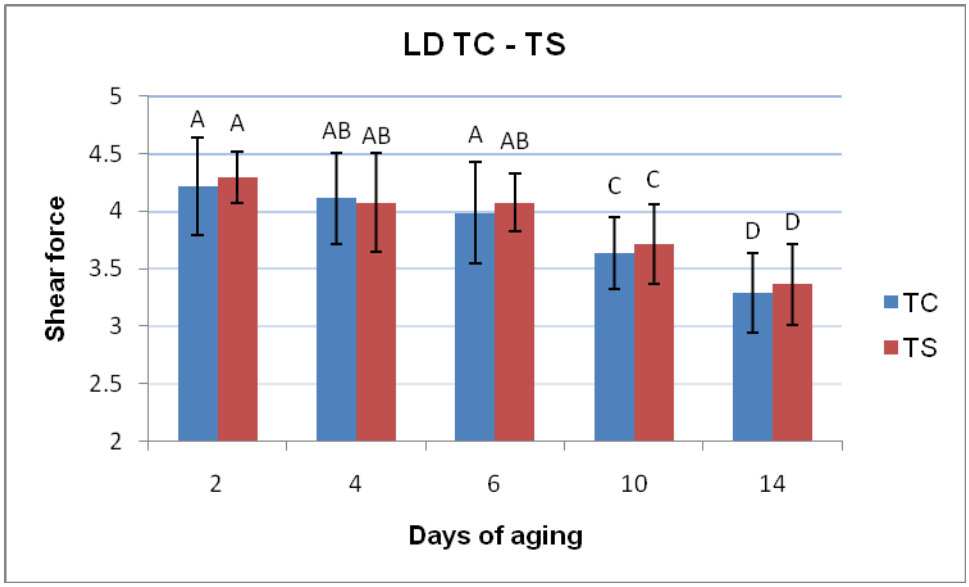


Figure 8.11. The shear force values of TS and TC treatments in the LD muscle over 14 days of aging with standard deviation

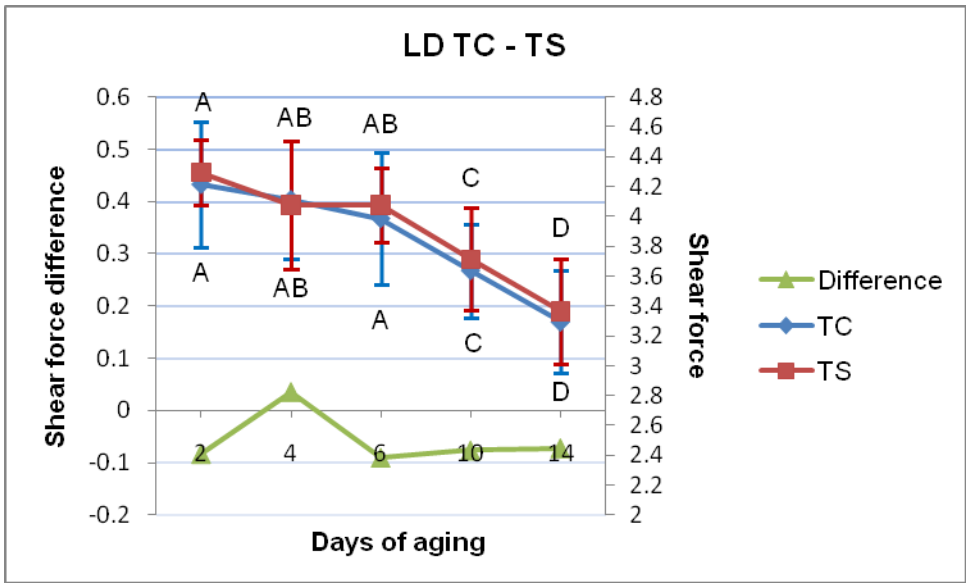


Figure 8.12. The difference in shear force between TS and TC treatments in the LD muscle over 14 days of aging with standard deviation

Discussion on tenderness

From the paired t-test analyses it became clear that although some differences were noticed in shear force for the TC method, none were significant for the GM muscle and those values that were significant in the LD muscle were only on the 4th day of aging. The difference in shear force for the significant value of TC in the LD muscle was only 0.3205 kg/ 1.27cm ∅. The TS method's largest difference was 0.2669 kg /1.27 cm ∅ in the LD muscle. This was on day 6 of aging. The fact that these differences, significant or not, were all smaller than 0.3205 kg/ 1.27cm ∅ suggests that the effects of these treatments are too little to be of value to the industry. According to literature (Eilers *et al,* 1995), a shear force value of 3.9 kg / 1.27 cm ∅ is seen as being tender. A shear force value of < 3.2 kg / 1.27cm ∅ is seen as being superior in tenderness. Due to the fact that the difference between these two classes is 0.5 kg / 1.27 cm ∅, we can speculate that a difference of 0.3205 kg / 1.27 cm ∅ would not be distinguished by a tasting panel. Even when the two treatments TS and TC were compared against each other, differences were < 0.2353 kg / 1.27 cm ∅ in the GM muscle and 0.0898 kg / 1.27 cm ∅ in the LD muscle. Although the differences in shear force between treatments becomes smaller as aging commences, it is not constant, which could imply that these small significant differences could be due to experimental variation.

Besides the fact that these differences were small, they were not consistently tougher or more tender for each treatment. From the 8 animals per treatment combination, not one treatment showed either an increase or decrease in tenderness for all 8 animals evaluated. When a treatment showed a mean positive more tender effect on the meat, it was the result of larger positive values from some of the 8 animals whilst the rest of the 8 animals showed a negative effect from the same treatment. With this the standard deviations from every treatment depicts how the shear force values overlap between treatments on the same day of aging showing how the treatment did not provide an positive effect per every animal. This was in contrast to the findings of Hostetler *et al* (1972) and Bouton *et al* (1973) who found a highly significant interaction ($P<0.001$) between hanging method and the eating quality of the meat. Bouton *et al* (1973) also reported decreased shear force for the *Gluteus medius* muscle. In his study the Tenderstretched meat was found to have unaged meat tenderness values equivalent to that found after 21 days of aging which was not the case for this study.

Form the various ANOVA (Tables 8.2 to 8.7) it is clear that there are significant differences in shear force between animals and very little between treatments. The Figures 8.1 to 8.12 depict how the difference in shear force between treatments either remain similar or become less as aging commences. In the LD muscle, significant differences were noticed on day 2 and 6 of aging for the TS method (Figure 8.8). From the figure it shows that the TS treatment had a more linear decline in shear force than its

control. These observations were made on the average shear force values for all animals and as previously stated do not mean that it was the norm for all animals and treatments. The fact that these differences were all below 0.3 kg / 1.27 cm ∅ raises the question whether this is viable for use on day 2 or 6, as both the treatment and the HS values are higher than 3.9 kg / 1.27 cm ∅ and is still considered as being less tender. Only after 10 days of aging, do the shear force values for both the TS and HS treatments reach between 3.9 and 3.2 kg / 1.27 cm∅. Both treatments are therefore seen as tender. However some significant differences were found they could be assumed irrelevant for the total shear force range was between 4.07 and 3.20 for the GM muscle and between 4.52 and 3.29 for the LD muscle which is very small. This means that these small differences between treatments, calculated as being significant, is not significant for the industry. These results are in contrast with that from Sorheim *et al* (2001) who found a reduction in LD shear force of 41% for TS hung carcasses and 23% for TC hung carcasses against their opposite normally hung sides. These increases in tenderness however were only documented when these carcasses were fast chilled. No effects were noticeable in slow chilling regimes. The main difference in this trial was that Sorheim did not use electrical stimulation, which may alleviate the effect of these two hanging methods.

A GLM procedure was run on the overall effect that the two treatments TC and TS had on the tenderness of the GM and LD muscles (Table 8.8 and 8.9 respectively). Over the 24 cattle used for this purpose, the mean values for all the TC and TS measurements were compared to all the measurements that were done on the normally hung HS sides. This was done while assuming all cattle were uniform and with the fact that all treatments were allocated randomly to the carcass sides. No differences (P = 0.9672) were found between these treatments within the GM muscle. Figure 8.13 shows the values per day of aging for the GM muscle and confirms the significant effect (P <.0001) that aging has on the tenderness of meat. Figure 8.14 gives a significant increase in tenderness (P = 0.0027) for TS and TC in the LD muscle on days 2, 4 and 10. On day 6 only TC was significantly different from its control.

From the overall effects of the three methods for all 24 animals (Table 8.8 and 8.9) no differences were noticed in the GM muscle. The LD muscle was clearly more affected however only before 14 days of aging. No treatment shifted the control from above 4 kg / 1.27 cm ∅ to below 3.9 kg / 1.27 cm ∅ on a constant basis. Therefore meat from either treatment on a specific day with shear force values > 3.9 kg / 1.27 cm ∅ and < 4.6 kg / 1.27 cm ∅ can be seen as slightly tender, whereas all the treatments < 3.9 kg / 1.27 cm ∅ and > 3.2 kg / 1.27 cm ∅ can be seen as tender. From this data it is clear that aging still has the most beneficial effect on the tenderness of meat.

Table 8.8. The anova for the 24 animals with treatments HS, TC and TS over 14 days of aging

Muscle=GM

The GLM Procedure

Dependent Variable: ShearForce

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	69	25.40237276	0.36815033	3.14	<.0001
	R-Square	Coeff Var	Root MSE	ShearForce Mean	
	0.576697	9.318814	0.342445	3.674769	
Source	DF	Type I SS	Mean Square	F Value	Pr > F
GrAnAgeFat	22	12.13258814	0.55148128	5.68	0.0002
Treatment	2	0.00649133	0.00324566	0.03	0.9672
Error(a)	18	1.74784933	0.09710274		
Day	4	9.62557638	2.4063941	20.52	<.0001
TrtxDay	8	0.39504926	0.04938116	0.42	0.9071
Error	159	18.64568597	0.11726847		
Corrected Total	228	44.04805873			

Gr = group; An = animal; Age = the age of the animal per classification; Fat = Fatness level of the animal

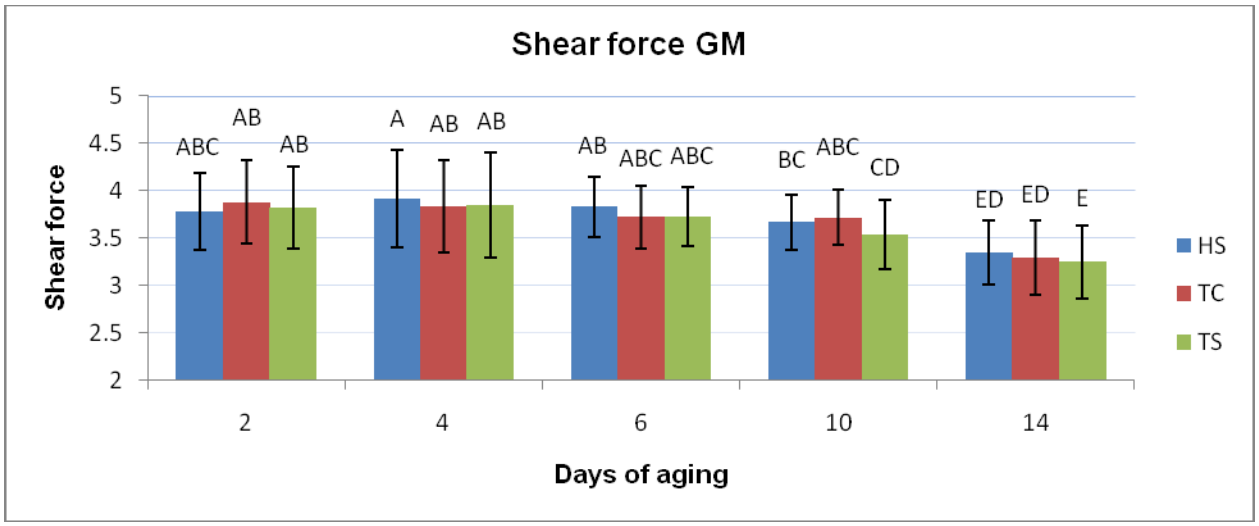


Figure 8.13. The shear force for each treatment on the selected days of aging in the GM muscle

Table 8.9. The anova for the 24 animals with treatments HS, TC and TS over 14 days of aging

Muscle=LD

The GLM Procedure

Dependent Variable: ShearForce

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	69	41.67975003	0.60405435	13.52	<.0001

R-Square	Coeff Var	Root MSE	ShearForce Mean
0.853634	5.368032	0.211342	3.937057

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GrAnAgeFat	22	13.71913217	0.62359692	9.45	<.0001
Treatment	2	1.10023516	0.55011758	8.34	0.0027
Error(a)	18	1.18757357	0.06597631		
Day	4	24.00808333	6.00202083	134.38	<.0001
TrtxDay	8	0.15742978	0.01967872	0.44	0.8952
Error	160	7.14650024	0.04466563		
Corrected Total	229	48.82625027			

Gr = group; An = animal; Age = the age of the animal per classification; Fat = Fatness level of the animal

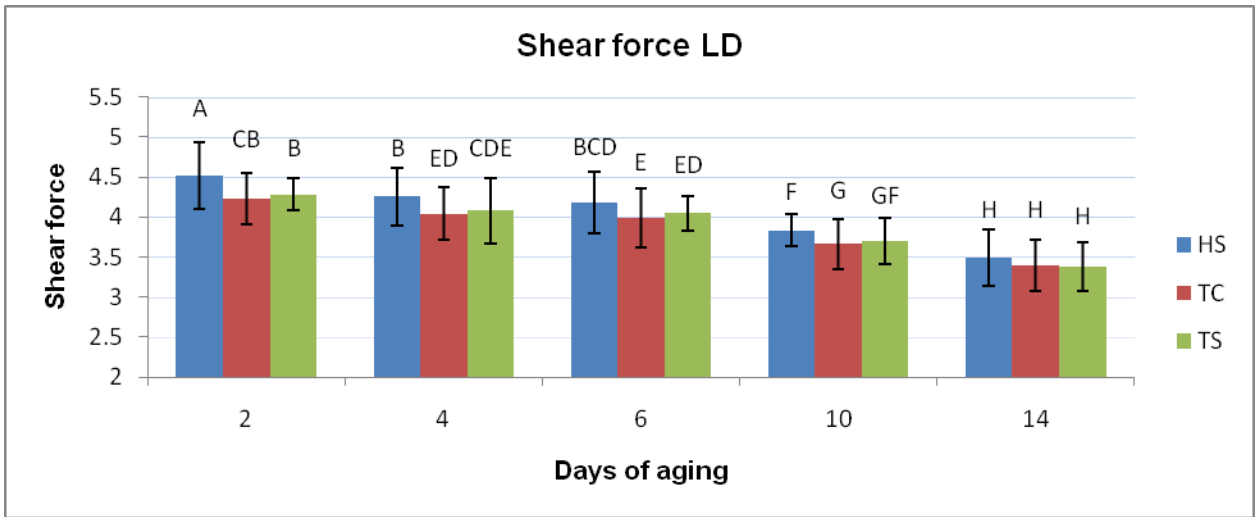


Figure 8.14. The Shear force for each treatment on the selected days of aging in the LD muscle with standard deviations

Although these treatments seemed to decrease the shear force of the LD muscle significantly, the difference in shear force were so small that it raises the question whether these significantly lower shear force values are of worth to the industry. A definite difference in tenderness can be seen through the effect of aging. In figure 8.14 it is shown how a treatment has no beneficial effect after 14 days of aging.

The studies from Derbyshire *et al* (2007) showed that although a hip suspended carcass had a significantly ($P< 0.05$) more tender *Longissimus* muscle compared to that of the Achilles hung carcass, the effect that ES or 7 days of aging or the combination of ES and 7 days of aging had on the hip suspended carcass was of no significant improvement. This concurred with the results of Dransfield *et al,* (1991) which found no significant benefit from combining ES with Tenderstretch. For this reason we can assume that the effects from TS or TC were dulled because of the use of ES and aging of 14 days.

Purge

The difference in purge between treatments is shown in Table 8.10. No significant differences were found in the pair t-test except between treatments TC and TS and only on days 2 and 14 of aging.

Table 8.10. Paired t-test of purge on combinations HS- TC, HS - TS and TC - TS

Purge											
Pair=HS-TC Muscle=GM						Pair= HS-TC Muscle=LD					
Day	N	Difference in Mean	Std Error	t Value	Pr > |t|	Day	N	Difference in Mean	Std Error	t Value	Pr > |t|
2	8	-0.0400	0.7478	-0.05	0.9588	2	8	0.3350	0.6780	0.49	0.6364
4	8	-0.6075	0.6736	-0.9	0.3971	4	8	0.5975	0.5387	1.11	0.304
6	8	0.2675	0.5264	0.51	0.6269	6	8	0.0825	0.3722	0.22	0.8309
10	8	-0.2775	0.9747	-0.28	0.7841	10	8	0.4950	0.3302	1.5	0.1776
14	8	-0.7100	1.4954	-0.47	0.6494	14	8	-0.7013	0.5867	-1.2	0.2709

Purge											
Pair=HS-TS Muscle=GM						Pair= HS-TS Muscle=LD					
Day	N	Difference in Mean	Std Error	t Value	Pr > |t|	Day	N	Difference in Mean	Std Error	t Value	Pr > |t|
2	7	-0.0871	0.6507	-0.13	0.8978	2	7	-0.0586	0.3742	-0.16	0.8808
4	7	-0.7843	0.5889	-1.33	0.2313	4	7	0.0229	0.5664	0.04	0.9691
6	7	-0.8200	0.5482	-1.5	0.1853	6	7	0.8229	0.6816	1.21	0.2728
10	7	-0.9600	1.2376	-0.78	0.4674	10	7	0.3800	0.5681	0.67	0.5285
14	7	-0.6900	1.1552	-0.6	0.5722	14	7	0.6200	0.7457	0.83	0.4376

Purge											
Pair=TC-TS Muscle=GM						Pair= TC-TS Muscle=LD					
Day	N	Difference in Mean	Std Error	t Value	Pr > |t|	Day	N	Difference in Mean	Std Error	t Value	Pr > |t|
2	8	-1.9050	0.5097	-3.74	0.0073	2	8	0.2600	0.7582	0.34	0.7417
4	8	1.6663	1.5783	1.06	0.3261	4	8	-0.3125	0.4881	-0.64	0.5424
6	8	-0.9938	0.8649	-1.15	0.2883	6	8	0.3700	0.4825	0.77	0.4682
10	8	0.7613	2.8855	0.26	0.7995	10	8	-0.9288	1.1282	-0.82	0.4375
14	8	-2.4475	0.9318	-2.63	0.0341	14	8	0.8938	0.3421	2.61	0.0348

From the ANOVAs on all the purge data for each treatment over all the animals, the following two figures are given over 14 days of aging. Within the GM muscle (Figure 8.15) the only significant difference was found on day 14 for the TS treatment. In the LD muscle (Figure 8.16), a difference in purge was noticed on day 14 for the TS treatment. Table 8.11 and 8.12 gives the ANOVA for purge between the treatments and days of aging for the GM and the LD muscles respectively.

Table 8.11. The anova for the 24 animals with treatments HS, TC and TS over 14 days of aging

Muscle=GM

The GLM Procedure

Dependent Variable: Purge

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	69	1126.225703	16.322112	7.43	<.0001

R-Square	Coeff Var	Root MSE	Purge Mean
0.76545	20.54921	1.482594	7.214846

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GrAnAgeFat	22	504.7507735	22.943217	6.54	<.0001
Treatment	2	46.3920879	23.196044	6.61	0.007
Error(a)	18	63.1268453	3.507047		
Day	4	478.495061	119.6237653	54.42	<.0001
TrtxDay	8	20.8287451	2.6035931	1.18	0.3117
Error	157	345.099366	2.198085		
Corrected Total	226	1471.32507			

Gr = group; An = animal; Age = the age of the animal per classification; Fat = Fatness level of the animal

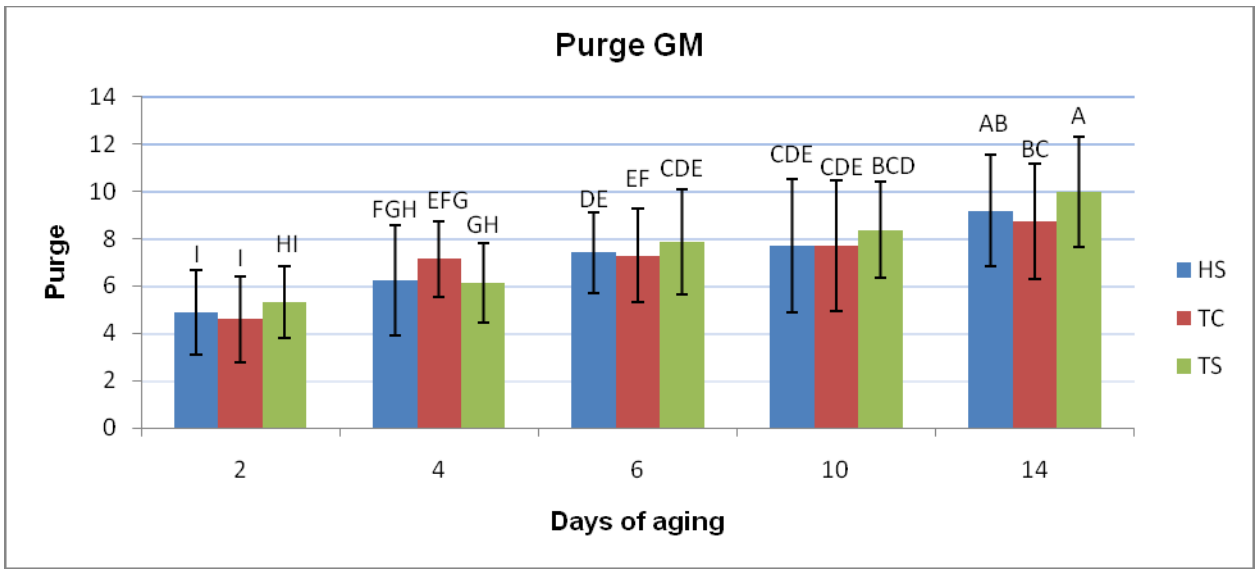


Figure 8.15. The Purge for each treatment on the selected days of aging in the GM muscle

Table 8.12. The anova for the 24 animals with treatments HS, TC and TS over 14 days of aging

Muscle=LD

The GLM Procedure

Dependent Variable: Purge

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	69	811.349688	11.758691	9.07	<.0001

R-Square	Coeff Var	Root MSE	Purge Mean
0.798439	23.39877	1.138566	4.865921

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GrAnAgeFat	22	573.9028667	26.0864939	18.39	<.0001
Treatment	2	5.2139948	2.6069974	1.84	0.1878
Error(a)	18	25.529469	1.4183038		
Day	4	174.3545232	43.5886308	33.62	<.0001
TrtxDay	8	4.9187325	0.6148416	0.47	0.873
Error	158	204.820418	1.296332		
Corrected Total	227	1016.170107			

Gr = group; An = animal; Age = the age of the animal per classification; Fat = Fatness level of the animal

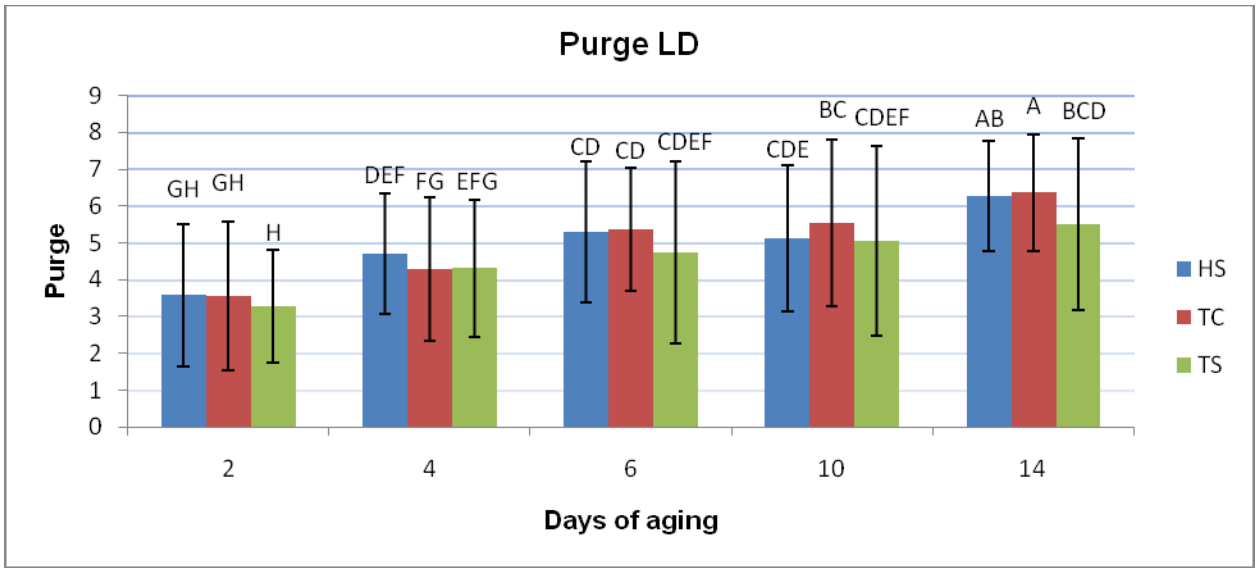


Figure 8.16. The purge for each treatment on the selected days of aging in the LD muscle

The most prominent differences in purge on the GM and the LD muscle were as a result of the amount of days the meat was aged. Purge increased to almost double the amount from two days of aging towards 14 days of aging. Standard deviations illustrate that the effect that treatment had on purge was inconsistent between days and of no significance.

Cooking loss

The paired t-tests on cooking loss for the combinations HS – TC, HS – TS and TC – TS, in the muscles GM and LD are depicted in Table 8.13. No differences were shown within the GM muscle although the LD muscle showed a difference on day 2 for the HS – TC and the HS – TS treatment combinations.

Table 8.13. Paired t-test of Cooking loss on combinations HS- TC, HS - TS and TC - TS

Cooking loss											
Pair= HS – TC Muscle=GM						Pair= HS – TC Muscle=LD					
Day	N	Difference in Mean	Std Error	t Value	Pr > |t|	Day	N	Difference in Mean	Std Error	t Value	Pr > |t|
2	8	-0.4750	0.4569	-1.04	0.3331	2	8	2.0538	0.5870	3.5	0.01
4	8	0.3113	0.3358	0.93	0.3848	4	8	0.4750	0.4106	1.16	0.2853
6	8	0.2275	0.5142	0.44	0.6715	6	8	-0.0738	0.4664	-0.16	0.8788
10	8	0.7438	0.8330	0.89	0.4016	10	8	0.0300	0.5238	0.06	0.9559
14	8	0.3275	0.6046	0.54	0.6048	14	8	-0.5875	0.8968	-0.66	0.5333

Cooking loss											
Pair= HS - TS Muscle=GM						Pair= HS - TS Muscle=LD					
Day	N	Difference in Mean	Std Error	t Value	Pr > |t|	Day	N	Difference in Mean	Std Error	t Value	Pr > |t|
2	7	-0.1886	0.6846	-0.28	0.7922	2	7	0.9443	0.3457	2.73	0.0341
4	7	-0.3814	0.6227	-0.61	0.5626	4	7	0.3786	0.1655	2.29	0.0622
6	7	0.2857	0.4123	0.69	0.5142	6	7	0.6500	0.3151	2.06	0.0847
10	7	-0.4757	0.5671	-0.84	0.4337	10	7	-0.2986	0.2687	-1.11	0.309
14	7	0.0057	0.7322	0.01	0.994	14	7	0.3443	0.7200	0.48	0.6495

Cooking loss											
Pair= TC - TS Muscle=GM						Pair= TC - TS Muscle=LD					
Day	N	Difference in Mean	Std Error	t Value	Pr > |t|	Day	N	Difference in Mean	Std Error	t Value	Pr > |t|
2	8	-0.5575	0.3964	-1.41	0.2024	2	8	-0.5050	0.5286	-0.96	0.3712
4	8	0.1225	0.3526	0.35	0.7385	4	8	0.1750	0.3233	0.54	0.6051
6	8	0.2725	0.3367	0.81	0.4449	6	8	-0.6125	0.3638	-1.68	0.1361
10	8	0.4313	0.2427	1.78	0.1188	10	8	-0.0325	0.3650	-0.09	0.9316
14	8	0.4113	0.4083	1.01	0.3474	14	8	0.0550	0.6750	0.08	0.9373

From the ANOVA on all the cooking loss data for each treatment over all the animals, the following two Tables and Figures are given over 14 days of aging. Within the GM muscle (Table 8.14 and Figure 8.17) the only significant difference was found on day 14 for the TS treatment. In the LD muscle (Table 8.15 and Figure 8.18), differences in cooking loss was noticed on day 2 between treatments and a slight difference for all muscles aged for 14 days.

Table 8.14. The anova for the 24 animals with treatments HS, TC and TS over 14 days of aging

Muscle=GM

The GLM Procedure

Dependent Variable: CookingLoss

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	69	336.9789119	4.8837523	2.19	<.0001

R-Square	Coeff Var	Root MSE	CookingLoss Mean
0.485269	4.57117	1.494653	32.69739

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GrAnAgeFat	22	273.4304748	12.4286579	9.09	<.0001
Treatment	2	0.1792396	0.0896198	0.07	0.9368
Error(a)	18	24.6161274	1.3675626		
Day	4	23.6725348	5.9181337	2.65	0.0353
TrtxDay	8	2.0380738	0.2547592	0.11	0.9987
Error	160	357.4381229	2.2339883		
Corrected Total	229	694.4170348			

Gr = group; An = animal; Age = the age of the animal per classification; Fat = Fatness level of the animal

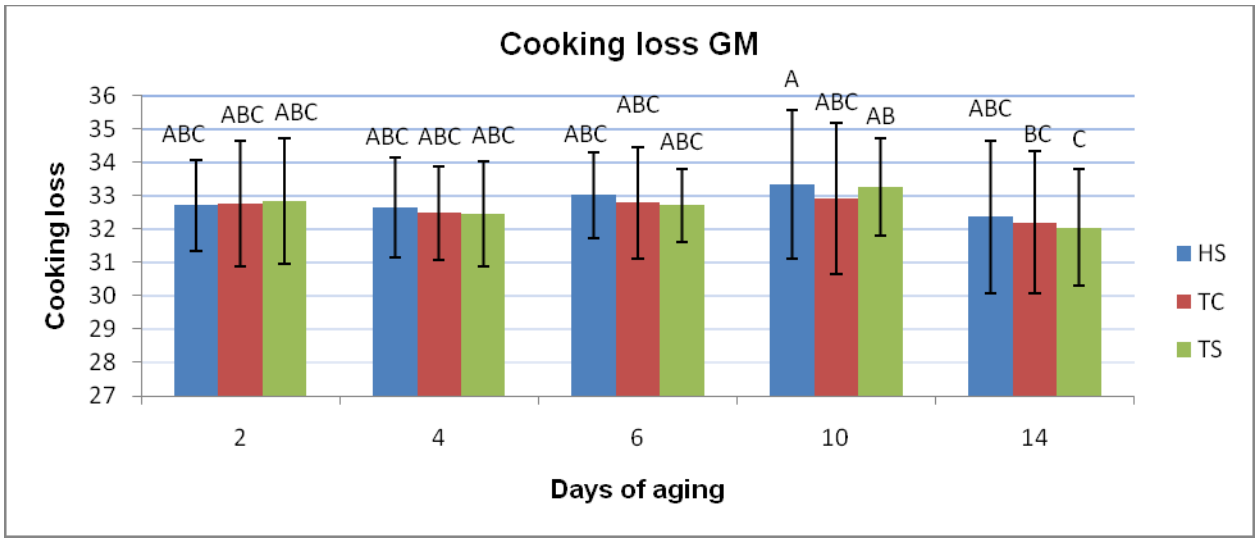


Figure 8.17. The Cooking loss for each treatment on the selected days of aging in the GM muscle with standard deviation

Table 8.15. The anova for the 24 animals with treatments HS, TC and TS over 14 days of aging

Muscle=LD

The GLM Procedure

Dependent Variable: CookingLoss

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	69	320.7068825	4.6479258	5.7	<.0001

R-Square	Coeff Var	Root MSE	CookingLoss Mean
0.713496	2.886616	0.902807	31.27561

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GrAnAgeFat	22	204.5708364	9.2986744	9.39	<.0001
Treatment	2	3.8875328	1.9437664	1.96	0.1695
Error(a)	18	17.8318051	0.9906558		
Day	4	25.6789315	6.4197329	7.88	<.0001
TrtxDay	8	18.9012757	2.3626595	2.9	0.0048
Error	158	128.7795316	0.8150603		
Corrected Total	227	449.486414			

Gr = group; An = animal; Age = the age of the animal per classification; Fat = Fatness level of the animal

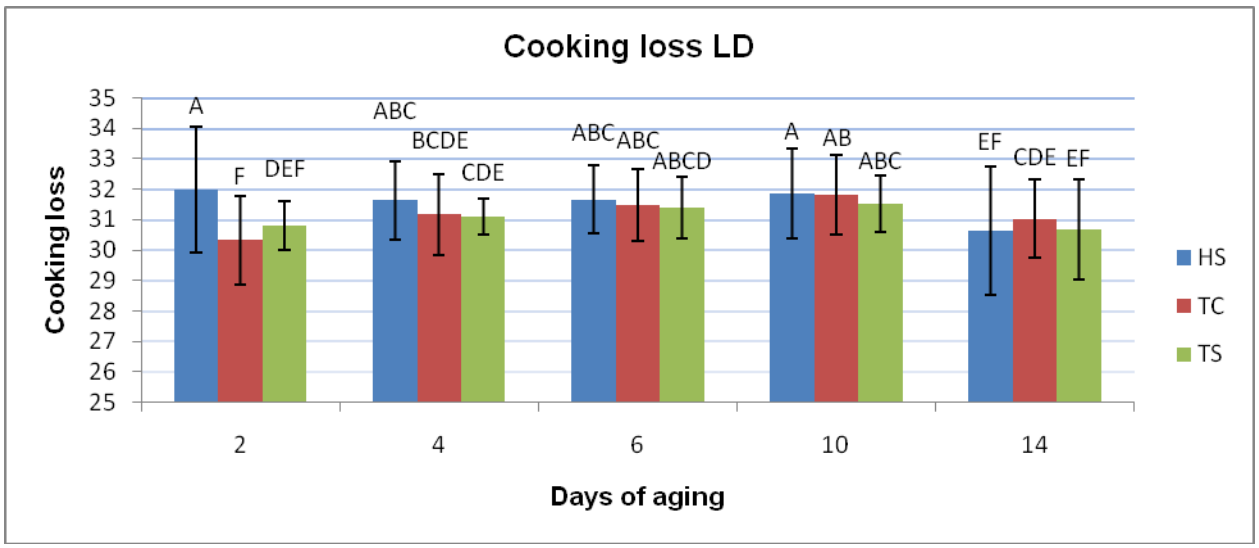


Figure 8.18. The cooking loss for each treatment on the selected days of aging in the LD muscle with standard deviation

Cooking losses were not significant between treatments and were all between 33.5 and 32 % for the GM muscle. Although some significant differences between treatments were noticed in the LD muscle (Figure 8.18) on day 2, the range between the highest and lowest values was only 32 % and 30.3 % respectively. The only relationship between the cooking loss and the two treatments are that some of the collagen fibres might realign which might increase juiciness and means less cooking loss.

Sarcomere length from main study

The average sarcomere lengths of all animals after 2, 6 and 10 days of aging, were related to their corresponding shear force values for each treatment on each muscle (Table 8.16). In addition to the fact that shear force of the GM and LD muscles became lower as aging of the meat continues, the sarcomere lengths increased during aging as well. The correlations between shear force and sarcomere lengths are depicted in Figures 8.19 and 8.20.

Table 8.16. The sarcomere length and their corresponding shear force values on day 2, 6 and 10 of aging for the GM and LD muscle

A 1 -24 GM

Days aged	2			6			10		
Treatment	HS	TC	TS	HS	TC	TS	HS	TC	TS
Shear force (kg/1.27cm)	3.776^{abc}	3.879^{ab}	3.818^{ab}	3.828^{ab}	3.719^{abc}	3.728^{abc}	3.665^{bc}	3.716^{abc}	3.533^{cd}
Sarcomere length (µm)	15.832^{a}	17.257^{b}	15.979^{a}	17.083^{b}	17.880^{c}	19.334^{d}	17.428^{c}	17.363^{c}	18.733^{d}

A 1 -24 LD

Days aged	2			6			10		
Treatment	HS	TC	TS	HS	TC	TS	HS	TC	TS
Shear force (kg/1.27cm)	4.518^{a}	4.225^{bc}	4.279^{b}	4.186^{bcd}	3.992^{e}	4.045^{de}	3.834^{f}	3.666^{g}	3.696^{fg}
Sarcomere length (µm)	18.637^{a}	18.308^{a}	20.234^{c}	19.001^{b}	19.004^{b}	21.993^{c}	18.932^{b}	19.075^{b}	20.732^{c}

The average sarcomere lengths were correlated to their corresponding shear force values for each treatment on each muscle. For the GM muscle (Figure 8.19), no significant correlation were calculated for any treatment, however it still shows that longer sarcomeres from the TS method had a slight tendency to be more tender although only 4% of this methods' significance is explained by the data.

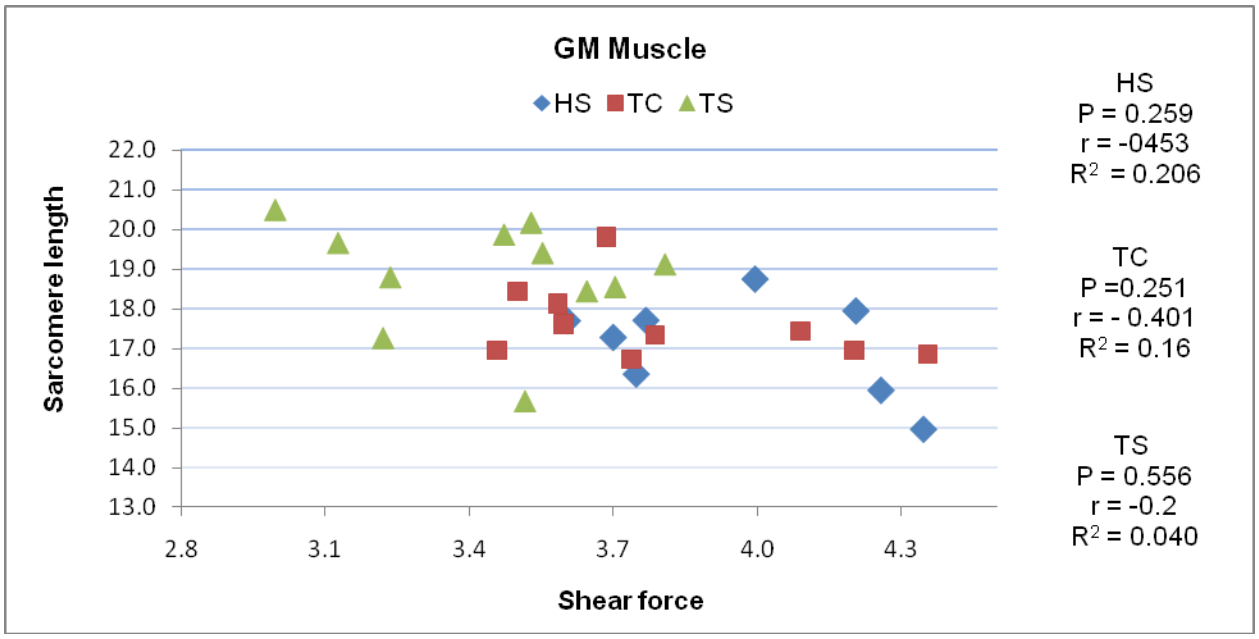


Figure 8.19. Correlation between shear force and sarcomere length in the GM muscle

In the LD muscle however, the TS method showed a significant (P = 0.009) correlation of r = -0.665 (Figure 8.20). However, only 44% of its significance is explained by the data (R^2 = 0.442). The correlations obtained from using the HS or TC interventions are inconclusive.

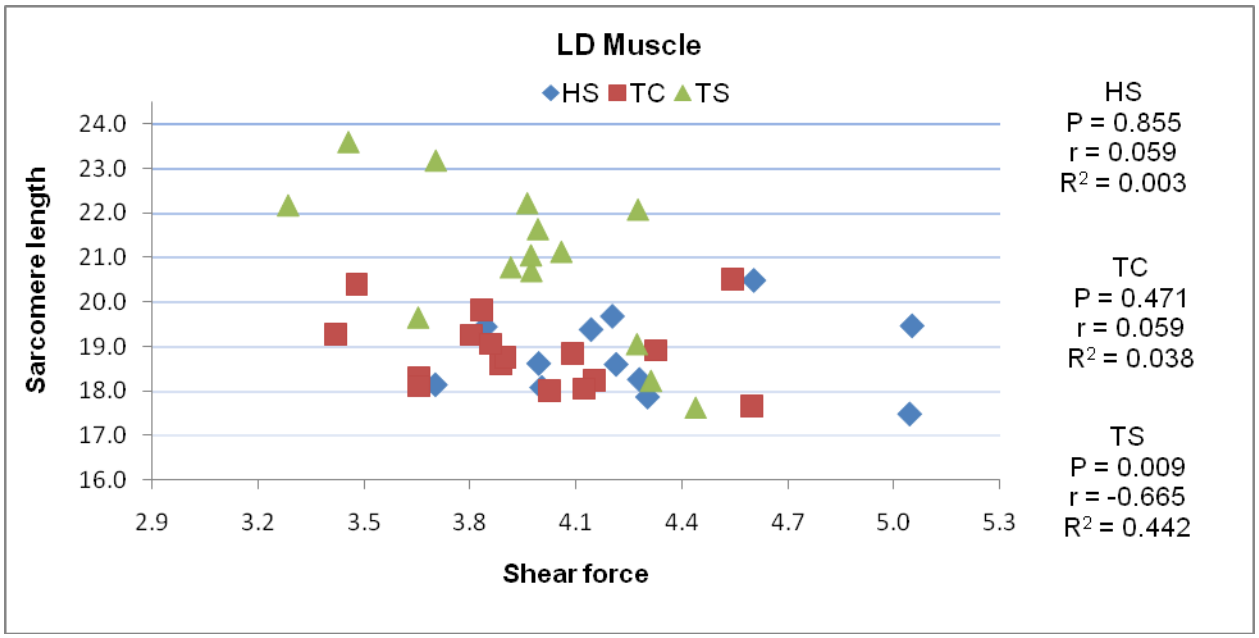


Figure 8.20. Correlation between shear force and sarcomere length in the LD muscle

Although the data showed that sarcomere lengths increased due to aging of the meat, the longest sarcomeres however did not indicate the lowest shear force values (Table 8.16). The correlations between sarcomere lengths and shear force were low for all the treatments except in the LD muscle where the TS method showed a higher correlation of r = -0.665 than the other treatments (Figure 8.19 and 8.20).

The fact that the correlation between longer sarcomeres and lower shear force values are low and inconsistent possibly indicates that sarcomere length does not have the sole influence on the level of shear force and that shear force is not directly linked to sarcomere length. Degradation of the fibres due to the calpain system also results in the elongation of sarcomeres from breakage of the Z-line. From the time the sarcomeres are degraded and the shear force values are influenced by the calpain system, the correlation between the two can become distorted. This was confirmed by Koohmaraie (1996) who noted that the relationship between sarcomere length and tenderness is strongly influenced by the degree of post mortem tenderisation whereas a very low relationship between sarcomere length and tenderness can be expected when the post mortem tenderisation is at a high level as well as the opposite, being a high relationship coinciding with low post mortem tenderisation.

Carcass Area

It has been postulated that using an intervention such as TS will result in the carcasses using more space in the cooler due to the new shape. To evaluate this, carcasses from each treatment were measured in height, width and cross section (side). The average cubic area for each treatment were calculated to be 1.04 m^3 for Tenderstretch, 0.89 m^3 for the Tendercut method and 0.81 m^3 for the normal Achilles hung carcasses (Tabel 8.17).

Table 8.17. The average carcass cubic area for the treatments TS, TC and HS

Treatment	Height (m)	Side (m)	Width (m)	Cubic Area m3
TS	2.10	1.24	0.4	1.04
TC	2.80	0.80	0.4	0.89
HS	2.55	0.80	0.4	0.81

By multiplying the cubic area used for each treatment with the number of carcasses to reach the same overall cubic area it is shown in Table 8.18 that 120 normally hung carcasses will make up a cubic area of 97.2 m^3. For the TS treatment, only 94 carcasses fitted into the same total area, whereas TC needed about a 109. From this we can speculate that chillers that are stacked to full capacity might not be able to do so when using the Tenderstretch intervention. Capacity is relative, as the data indicates. Carcasses having the Tenderstretch technology would therefore limit the numbers of carcasses that would fit into the chiller.

Table 8.18 The amount of carcasses from each treatment that fits into the same cubic area

Differences between treatments			
Treatment	Carcasses	Cubic area/carcass	Total area
HS	120	0.81	97.2
TS	94	1.04	97.76
TC	109	0.89	97.01

Conclusion

Part of the reason for the lack of positive results in this study could be due to the fact that all the shear force measurements were done on steaks cooked to an internal temperature of 70 °C. From literature it was found that the effect of stretching only comes into effect at 80°C where the thermal contraction is greater than at 60 °C. As mentioned by Rowe (1974), the collagen fibres partly unfolds when the muscle is stretched pre-rigor and therefore is more oriented in the direction of the fibres, which means more collagen fibres being cut in the perpendicular shearing of the muscle fibres. When collagen only starts denaturing at temperatures above 60 °C, the full effect of the hanging methods could not yet have been revealed at temperatures of 70 °C such as it could have been at 80 °C. The question posed is whether the consumer requires softer steaks at 65 °C, which are labeled medium rare, or softer steaks at higher temperatures (80 °C) where the collagen fibers starts to degrade, nevertheless leading to tender meat as well.

When stretching methods were combined with electrical stimulation (ES), only an insignificant decrease in shear force of 0.28 kg / 1.27 cm ∅ was found (Derbyshire *et al*, 2007). This concurs with the current study where increases in tenderness were also insignificant (values of < 0.2855 kg / 1.27 cm ∅). The ES system used in this specific trial (45V @ 17Hz, pulse width 5 m/s for 45 seconds) can therefore be seen as sufficient in lowering the pH at the optimum temperature. Dransfield *et al* (1991) also found no beneficial improvement from combining stretching methods and ES. Derbyshire *et al* (2007) concluded that the effect that ES and 7 days of aging or the combination of Stretching and 7 days of aging, provides the same effect as these stretching methods and that the combination of all three variables (ES, 7 days aging and stretching) does not improve the eating quality of the meat.

From the current study, when ES is correctly applied and all the optimum chilling regimes are implemented in such a way that the correct pH is obtained at the optimum temperature for optimal enzymatic degradation, there is no need for different carcass suspension methods. When combining this with the lower number of carcasses that can fill a chiller to capacity, when using TS, the use of these methods becomes unnecessary.

Chapter 9

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