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Pinning Vs ageing

Assessment of effect of blade tenderisation on microbial load and structure of selected subprimals

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Executive summary

Mechanical tenderisation (blade tenderisation, or 'pinning') is used to improve the tenderness and thus palatability of low value cuts, e.g. steaks from silverside, topside or knuckle. This report describes an investigation of the effect of pinning on tenderness of selected whole muscles from each of these cuts, as determined by objective measurement of shear force; the effect of pinning on cooking losses; and the potential for microbial contamination on the surface of the muscle to be transferred to the deep tissues.

M. biceps femoris, *M. semimembranosus* and *M. rectus femoris* were dissected out from primal cuts, 24 hours after slaughter. A cocktail of *Escherichia coli* was painted onto the surface of the muscles and allowed to dry before the muscles were passed through a Ross tenderiser. The muscles were then quartered and each quarter assigned to 0, 7, 14 or 21 days of vacuum-packaged storage.

Tenderness
Peak shear force findings were different for each of the muscles investigated. For Control, non-pinned muscles, *M. semimembranosus* showed a highly significant improvement in tenderness over the ageing period whereas *M. rectus femoris* was little changed by ageing. There was some evidence of improved tenderness with ageing of *M. biceps femoris* but the effect was not as clear as with *M. semimembranosus*.

Except for *M. biceps femoris*, the other muscles investigated did show an improvement in tenderness following pinning, but generally the magnitude was low compared with the effect of ageing. Neither ageing nor pinning had any significant affect on the contribution of connective tissue to overall texture.

Cooking losses
Pinning did not significantly affect weight losses when cooked at either 70 or 80°C.

Microbiology
Microbiologically, pinning transferred microorganisms from the surface of the primal to the deep tissue. The proportion transferred ranged from zero to 63%, but on average was around 3-5%. If pathogens were present on the surface of the primal, they would be expected to be present at very low levels, so the numbers migrating into deep tissue would be very low. Thus, a USDA risk assessment concluded that the risk of illness from blade tenderised meat is only marginally greater than the risk of illness from non-blade tenderised meat.

Microbial counts in deep tissue declined during storage at 0°C under vacuum packaging.

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1 Introduction

Tenderness is the attribute most desired by consumers when eating a beef steak (Cowan *et al.* 1999, Miller *et al.* 2001). The primal cuts that are rated the highest for tenderness generally command the highest price and are normally consumed as steaks that are grilled or fried. The meat from lower value primal cuts is recommended to be wet-cooked before consumption or ground to improve its palatability but is usually sold at a lower price than meat for steaks or roasts.

Most of the muscles from the beef butt are generally considered unsuitable for use as steaks. This is mainly due to their inherent poor rating for tenderness, which in turn is related to the amount of connective tissue in the muscle. The greater the connective tissue content of the muscle, the less tender the meat produced. In an evaluation of 40 bovine muscles, Belew *et al.* (2003) rated the *M. semimembranosus*, the *M. semitendinosus*, *M. vastus lateralis* and the *M. adductor* amongst the least tender as measured by the Warner-Bratzler shear force. Even after 28 days ageing these muscles, along with the *M. biceps femoris*, rated poorly for tenderness (Gruber *et al.* 2006). Meat from older animals is also less tender than meat from young animals (Berry *et al.* 1974a, Tatum *et al.* 1978). A number of factors could account for this, including increased muscle fibre thickness, decreased sarcomere length, or the fact that the connective tissue forms firm cross-links within the muscle as the animal matures (Cooper *et al.* 1968, Covington *et al.* 1970, Berry *et al.* 1974b, Taylor 2004). A consumer study by Dunsing (1959) found that consumers preferred beef from younger animals as it was more tender.

The major commercial cuts from the butt are the topside, silverside and the knuckle. The *M. semimembranosus* and *M. adductor* are the major muscles of the topside (AUS-MEAT 1998) and are not considered suitable for grilling or pan frying (MSA 2004). The *M. biceps femoris* and *M. semitendinosus* form the major portion of the silverside (AUS-MEAT 1998) and are normally prepared for Australian domestic consumption by corning. The major muscles of the knuckle are the *M. rectus femoris*, *M. vastus medialis*, *M. vastus intermedius* and the *M. vastus lateralis* (AUS-MEAT 1998). The only one of these muscles considered by MSA (2004) to be suitable for grilling is the *M. rectus femoris*. This muscle is also rated higher for tenderness by Belew *et al.* (2003) than most of the other muscles of the butt.

The response of these muscles to ageing is variable. The *M. biceps femoris* (silverside) achieves most of its ageing tenderness in about one week but improves very little in tenderness, whereas the *M. vastus lateralis* and *M. vastus intermedialis* from the knuckle continue to improve in tenderness for up to three weeks (Gruber *et al.* 2006). However, even after 28 days ageing as steaks, Gruber *et al.* (2006) ranked most of these muscles amongst the least tender from a beef carcase. Improving tenderness by other means such as mechanical tenderisation or 'enhancement' by injection with various salt solutions would therefore appear an inviting proposition for these primal cuts.

Mechanical tenderisation by insertion of a bank of blades into the meat ('pinning' or 'needling') has been used to achieve a more acceptable product from these lower value primals and sub-primals and to improve the palatability of meat from older animals. Blade tenderisation improves tenderness by disrupting the muscle structure with thin blades that penetrate the muscle normally as a primal cut. This milestone report details investigations into the effect of single-pass blade tenderisation on objective measurement of tenderness of some hindquarter muscles, and the potential for microorganisms to be internalised during the pinning operation.

2 Methods

2.1 Sample collection

2.1.1 Trial 1

The objective of the first trial was to investigate the transfer of microbial contamination from the surface of beef primals to the interior of the primal as a result of the pinning process. Three topsides, four silversides and three rounds were used for the trial. These had been stored under vacuum at 0°C for two weeks prior to the trial. A mixed culture of Nalidixic Acid resistant *E. coli* K12, and non-antibiotic resistant *E. coli* K12 was prepared in Tryptone Soy Broth (TSB; Oxoid CM0876), to a level of 8.8 $\log_{10}$ cfu/mL. The organism was grown overnight at 37°C immediately before use. This culture was applied to the upper surface of each primal cut, using a sterile sponge (MicroSponge®; Biomerieux). The primal cut was then left on the bench for 20 minutes, inoculated surface uppermost, to dry, before being passed once through the Ross Tenderiser machine (figure 1). This machine has a bank of 5mm wide blades positioned at tight angles to one another, which are mechanically inserted into the meat (figure 2), resulting in 32 incisions per square inch. Care was taken to ensure that the primals were placed into the machine with the inoculated surface uppermost. Following pinning, a 10 cm² excision sample was taken aseptically from the upper surface of the primal, using a cork borer. The primal was then turned over to show the bottom, un-inoculated surface. This was wiped with 70% ethanol and sliced into on a vertical plane, stopping approximately two-thirds of the way through the primal. Care was taken to ensure that the knife did not pass through the inoculated surface of the primal. The interior of the primal thus exposed was turned uppermost, and re-swabbed with 70% ethanol. A window measuring approximately 7 cm x 7 cm was dissected out from this swabbed area, and the deep muscle tissue thus exposed sampled using a sanitised cork-borer and sterile scalpel blade. An area of 10 cm² and 3 mm deep was removed as the deep muscle sample.
The primals were then vacuum packed and stored at 0°C for 28 days, after which a further deep muscle sample was collected as described above.

Figure 1: Ross tenderiser

Figure 2: Tenderising a silverside

2.1.2 Trial 2

Trial 2 aimed in part to supplement the microbiological data gathered during trial 1: by repeating the procedure, and also taking samples of deep muscle at days 7, 14 and 21 of storage; and to investigate the effects of pinning and subsequent ageing on the objective measurements of cook loss and tenderness. Four silversides, four topsides and four knuckles were purchased from a local processor. primals were delivered to Food Science Australia 24 hours after slaughter. They had been chilled overnight and boned in the morning prior to delivery. Each was seamed to isolate the largest subprimal from each cut: *M. biceps femoris*, *M. semimembranosus* and *M. rectus femoris*. One subprimal of each type was used as the control cut: non-inoculated and not pinned. The other three subprimals were inoculated with marker organism as described above, and passed once through the Ross tenderiser. Each subprimal was then sectioned into four quadrants. One quadrant was used for the day zero samples; the remaining quadrants were individually vacuum packaged and stored at 0°C for 7, 14 and 21 days. On day zero, an excision sample was taken as described above from the upper surface and deep tissue of each subprimal, and at each subsequent sampling point, from the deep tissue. The remaining tissue was utilised for the objective tests.

2.2 Microbiological analysis

Each sample was refrigerated after collection and processed within 4 hours of collection. Briefly, 50 mL of 0.85% saline was added to each excision sample, this was stomached for 2 minutes, and a decimal dilution series prepared in saline. The dilutions were plated onto Petrifilm® APC plates (3M), Petrifilm® coliform plates (3M) and Violet Red Bile Glucose Agar (VRBG; Oxoid CM0485) plates containing 200ppm Nalidixic The Acid (VRBG-Nal). The Pertrifilm APC plates

were incubated at 30°C for 72 hours, and the Petrifilm coliform and VRBG-Nal plates at 37°C for 24 hours. Colonies were counted and counts per square centimetre calculated.

2.3 Cooking procedure and cook loss

Samples of uniform shape and size (approximately 100 g) were weighed then placed in plastic bags and immersed in a water bath at 70°C or 80°C for 1 hour. The bags were cooled for 10 minutes under running tap water (20°C). The juice was drained off and the meat gently blotted dry and re-weighed. Cook loss was determined by mass difference, and expressed as the percentage of loss relative to the initial weight.

2.4 Objective measurements

Assessments of meat texture were made using the Warner-Bratzler (WB) shear force measurement on samples cooked at both 70°C and 80°C for 60 minutes, using a Lloyd Instruments LRX Materials testing machine fitted with a 500 N load cell (Lloyd Instruments Ltd., Hampshire UK).

Following overnight storage at 4°C, the cooked samples were cut into sub-samples for textural analysis. Sample thickness, shape and fibre orientation were cut following the protocols outlined in Bouton *et a*l. (1971) and Bouton and Harris (1972). Six sub-samples having a rectangular cross-section of 15 mm wide by 6.7 mm deep (1 cm^2 cross-sectional area) were cut from each sample, with fibre orientation parallel to the long axis, and at right angles to the shearing surface. The force required to shear through the clamped sub-sample with a 0.64 mm thick blade pulled upward at a speed of 100 mm/min at right angles to fibre direction was measured as shear force. This allowed the determination of peak force (PF), initial yield (IY), and peak force minus initial yield (PF-IY). The mean ± standard deviation is presented in all Figures.

2.5 Statistical analysis

Results were entered into an Excel (Microsoft Inc) spreadsheet and analysed by analysis of variance (ANOVA) using MINITAB statistical software (Minitab Inc, PA).

3 Results

3.1 Microbiological analysis

3.1.1 Trial 1

Following inoculation and drying, the surface microbial loads of the primals (figure 3) were mean APC 6.18 log_{10}cfu/cm² (range 5.42 – 7.1) and mean coliform count (TCC) 4.43 log_{10}cfu/cm² (range 3.3 – 5.88). In general, counts were highest on the topside, and lowest on the round, although this trend was not statistically significant (figure 4). After pinning, the deep tissues carried mean APC of 2.58 log_{10}cfu/cm² (range below detection limit – 3.85), and mean TCC of 1.86 log_{10}cfu/cm² (range below detection limit – 3.28). In terms of the proportion of organisms transferred from the surface of the primals to the deep tissue, mean 0.13% (range 0 – 0.4%) of the surface APC was present in the deep tissue, while mean 6.86% (range 0 – 63.3%) of the TCC was transferred. In one sample the percentage TCC transferred was 63.3%. This high count may have resulted from cross-contamination at sampling, as all other percentages transferred were 10% or below. When this aberrant sample was removed from the calculation, the mean TCC transferred to deep tissue was 1.90%.

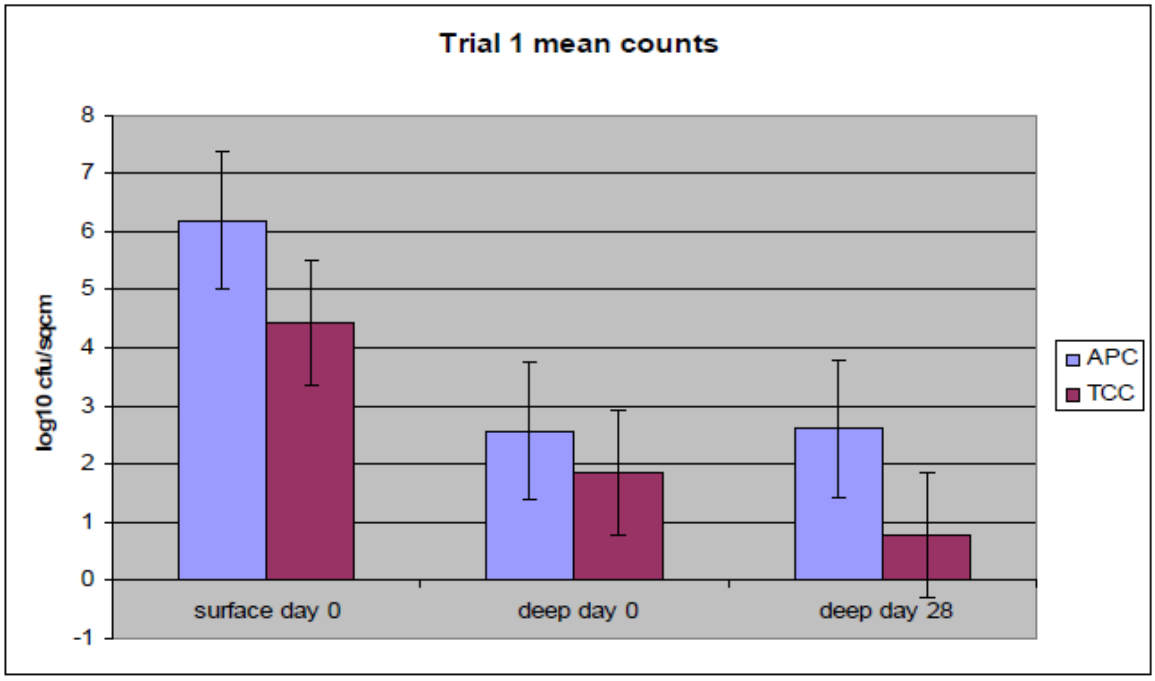


Figure 3: Mean microbial load on all primals before pinning (surface), after pinning (deep day 0), and after 28 days storage under vacuum (deep day 28)

Following 28 days of storage under vacuum, there was little difference in deep tissue APC (mean 2.62 log_{10}cfu/cm²; range 1.97 – 3.8), while TCC had dropped to mean 0.79 log_{10}cfu/cm² (range below detection limit – 2.44). This decline was not statistically significant. In general, the microbial load in deep tissue declined over the 28 days of storage in all primals, although a slight non-significant increase was observed in APC in rounds (figure 4).

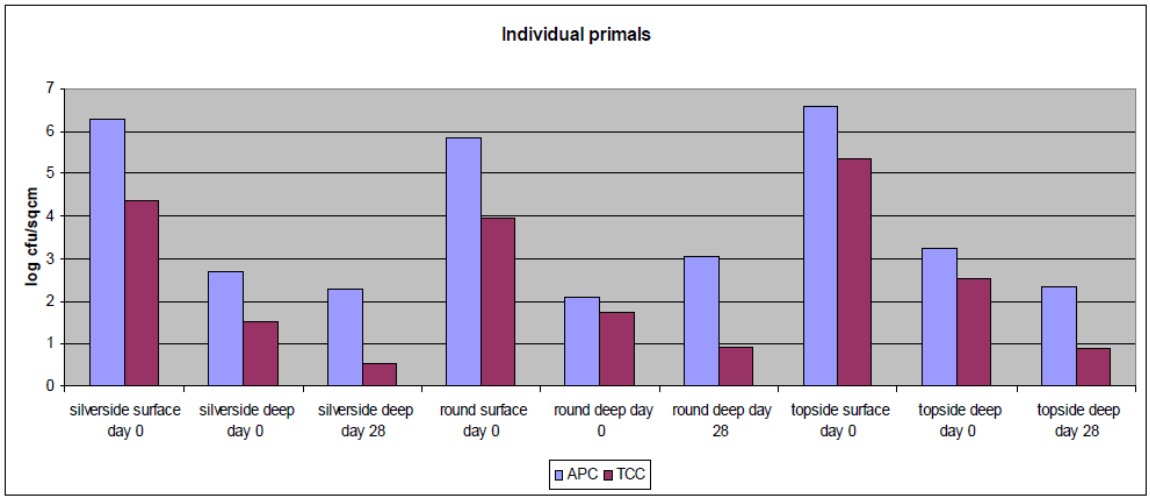


Figure 4: Microbial load of different primals (silverside, round and topside) before pinning (surface), after pinning (deep day 0), and after 28 days storage under vacuum (deep 28)

3.1.2 Trial 2

The control, uninoculated primals had a mean surface APC of 3.23 log_{10}cfu/cm² (range 2 – 4.08), and mean surface TCC of 3.39 log_{10}cfu/cm² (range 2.3 – 4.02) (figure 5). Deep tissue samples yielded no coliforms, and one muscle, the *M. biceps femoris*, yielded 0.6 log_{10}cfu/cm² APC. Inoculated primals had a mean surface APC of 5.87 log_{10}cfu/cm² (range 3.88 – 7.3), and TCC of 5.68 log_{10}cfu/cm² (range 3.4 – 7.3). Mean surface marker *E. coli* was 0.43 log_{10}cfu/cm² (range below detection limit – 1.18). Nalidixic acid resistant *E. coli* was recovered from the surface of one of three of each of *M. biceps femoris* and *M. semimembranosus*, and from two of three *M. rectus femoris*

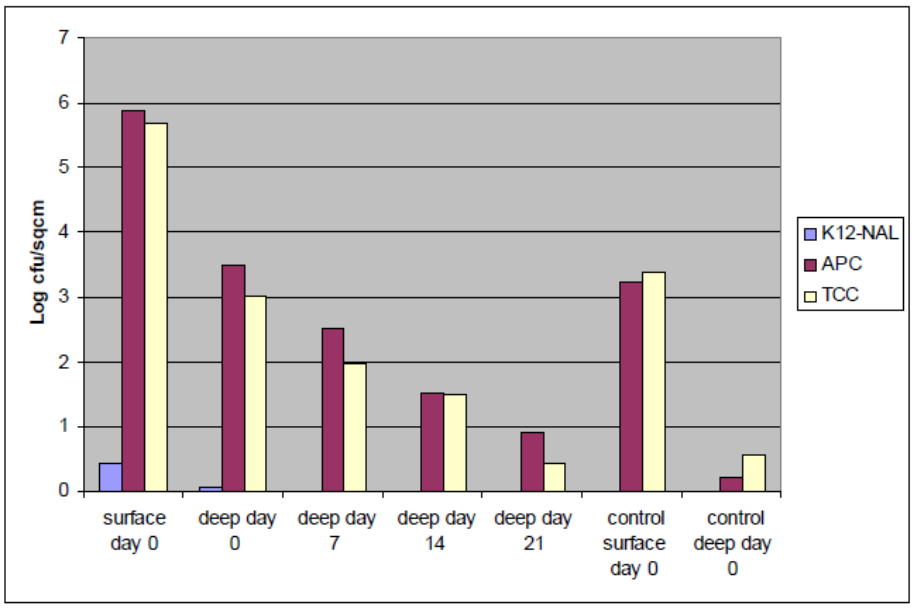


Figure 5: Mean microbial load of inoculated pinned primals and uninoculated controls on the surface and deep tissue after 7, 14 and 21 days of storage

Following pinning, Nalidixic acid resistant *E. coli* was recovered from two deep tissue samples only: a *M. semimembranosus*, from which the organism had not been recovered from the surface, and a *M. rectus femoris* from which the surface sample yielded 0.7 log_{10}cfu/cm². These were at a level of 0.7 and 1.54 log_{10}cfu/cm² respectively. Deep tissue mean APC across all muscles was 3.50 log_{10}cfu/cm² (range 2.02 – 5.4), and TCC 3.40 log_{10}cfu/cm² (range 2.3 – 5.31). In terms of the proportion of surface contamination transferred to the deep tissues during pinning, on average, 2.88% of surface APC and 5.5% of surface TCC was found in the deep tissues (ranges 0 – 20% and 0 – 51.25% respectively). Again, there was one sample (*M. rectus femoris* 4) which demonstrated a substantially higher transfer rate than the others (20% of APC, compared with 6.3% or less; and 51.1% of TCC, compared with 10% or less). This piece of muscle was very small, and it is possible that cross-contamination could have occurred during sampling. When this sample was removed from the calculation, mean APC transfer was 1.33%, and mean TCC transfer was 1.36%.

During ageing, the microbial counts in the deep tissue fell off steadily in all muscles (figure 6). By day 7, mean APC was 2.51 log_{10}cfu/cm² (range 1.4 – 3.74) and TCC 1.97 log_{10}cfu/cm² (range below detection limit – 3.02); by day 14 mean APC was 1.52 log_{10}cfu/cm² (range below detection limit – 4.35) and TCC 1.49 (range below detection limit – 4.08); and by day 21 mean APC was 0.92 log_{10}cfu/cm² (range below detection limit – 4.04) and TCC 0.44 log_{10}cfu/cm² (range below detection limit – 3.38). By day 21, two of three *M. biceps femoris*, two of three *M. rectus femoris* and one of three *M. semimembranosus* gave APC below detection limit (<0.7 log_{10}cfu/cm²), and only one *M. rectus femoris* gave a TCC reading (3.98 log_{10}cfu/cm²) above detection limit (<0.7 log_{10}cfu/cm²).

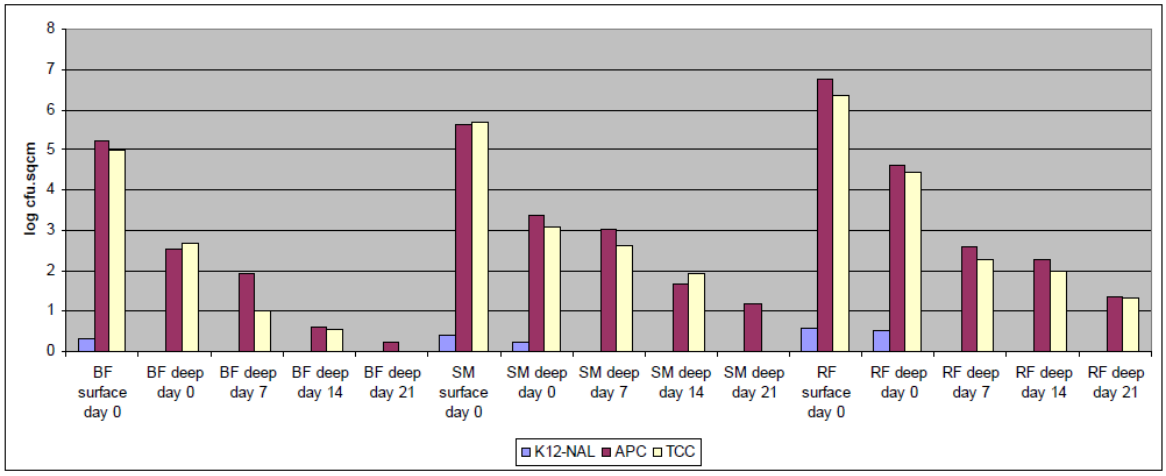


Figure 6: Mean microbial counts by muscle type after 0, 7, 14 and 21 days storage. BF, SM and RF refer to *M. biceps femoris*, *M. semimembranosus* and *M. rectus femoris* respectively

3.2 Effect of pinning on meat quality attributes

3.2.1 Objective measurements of tenderness

The effect of pinning on tenderness was determined using the Warner-Bratzler method to measure shear force on raw and cooked (70°C or 80°C) muscle samples. Two temperatures were used to relate the outcomes to 'medium' and 'well done' degrees of cooking. However, although the lower cooking temperature generally resulted in more tender meat the variability within samples and treatments was greater for those samples cooked at the higher temperature.

Texture measurements on raw muscles

For all raw samples, irrespective of muscle type, the peak force shear values obtained were low (figures 7 – 9) compared with cooked samples (figures 10 – 12) and did not show any significant differences between treatments. Lu and Chen (1999) reported that Warner-Bratzler shear force measurements were not good indicators for predicting tenderness of raw beef muscle.

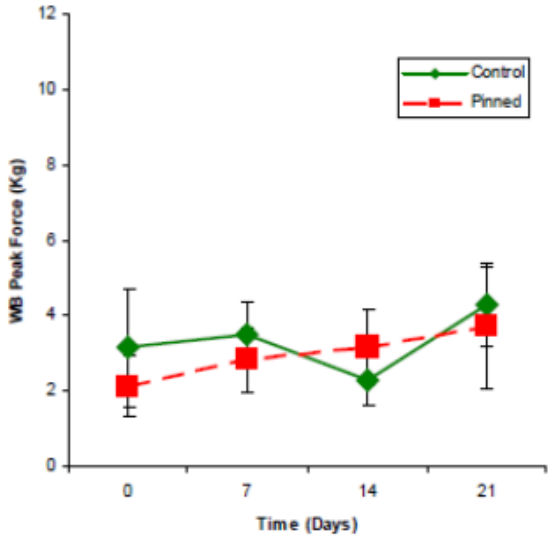


Figure 7: Effect of pinning and ageing on the texture (peak shear force) of raw *M. biceps femoris*

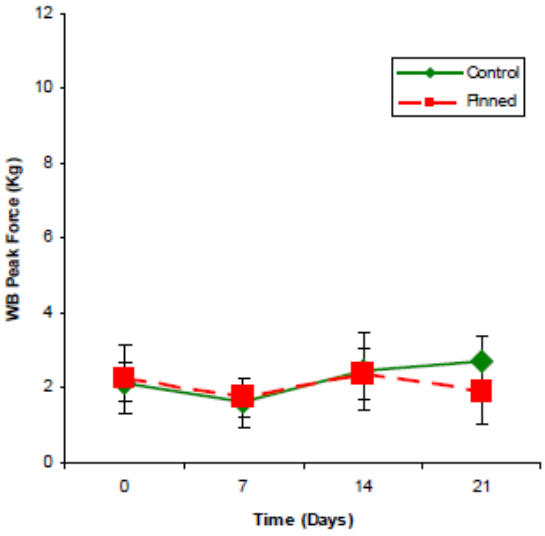


Figure 8: Effect of pinning and ageing on the texture (peak shear force) of raw *M. semimembranosus*

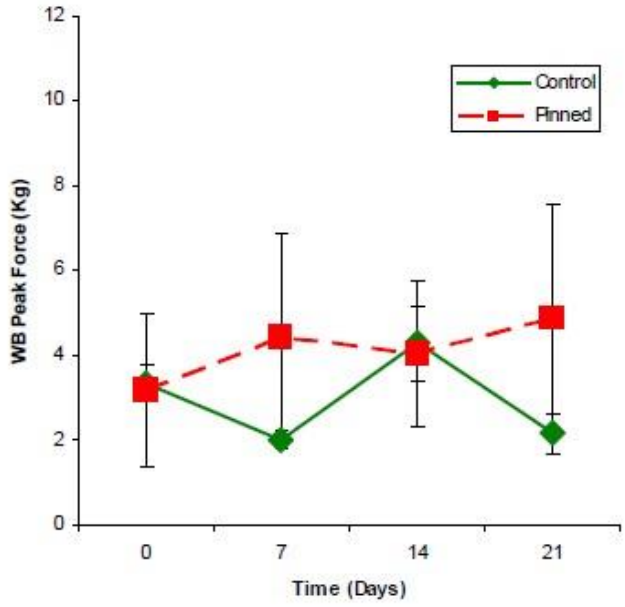


Figure 9: Effect of pinning and ageing on the texture (peak shear force) of raw *M. rectus femoris*

Texture measurements on cooked muscles

M. biceps femoris

Ageing of control (not pinned) *M. biceps femoris* at 0°C resulted in a significant improvement in tenderness within 7 days of muscles cooked at 70°C which continued to improve until Day 21 (figure 10). Peak shear force values were reduced by about 34% within 7 days and by 45% at Day 21. Any benefit of pinning on tenderness was most obvious on Day 0 where pinning significantly (*P<0.001*) reduced shear force by more than 2 kg. Muscles having peak force values of about 7 kg would be considered 'tough' and therefore an improvement to 5 kg or lower, would make the meat acceptable in terms of tenderness. Although the differences between treatments at Day 7 and Day 14 were not different, compared with the control, the pinned samples were less tender (P<0.05) at Day 21. This may be an irregularity as the shear value actually increased at this time. Also, this was not observed when the meat was cooked at 80°C rather than 70°C (figure 10, bottom).

Cooking muscles at 80°C instead of 70°C generally produces meat that is less tender (higher shear values) but this was not so obvious with *M. biceps femoris* (figure 10). The effect of ageing on tenderness of Controls was not apparent until Day 14 when these muscles were cooked at 80°C. However, compared with Controls, pinning significantly reduced (*P<0.001*) peak shear force on Days 0 and 7 with the trend continuing to the end of the trial. It is evident that with *M. biceps femoris* that pinning improves tenderness to such an extent that muscle ageing for 21 days contributes no further improvement.

It is evident from the Peak Force minus Initial Yield (PF-IY) data that neither ageing nor pinning had any significant effect on the contribution of connective tissue to overall texture (figure 10).

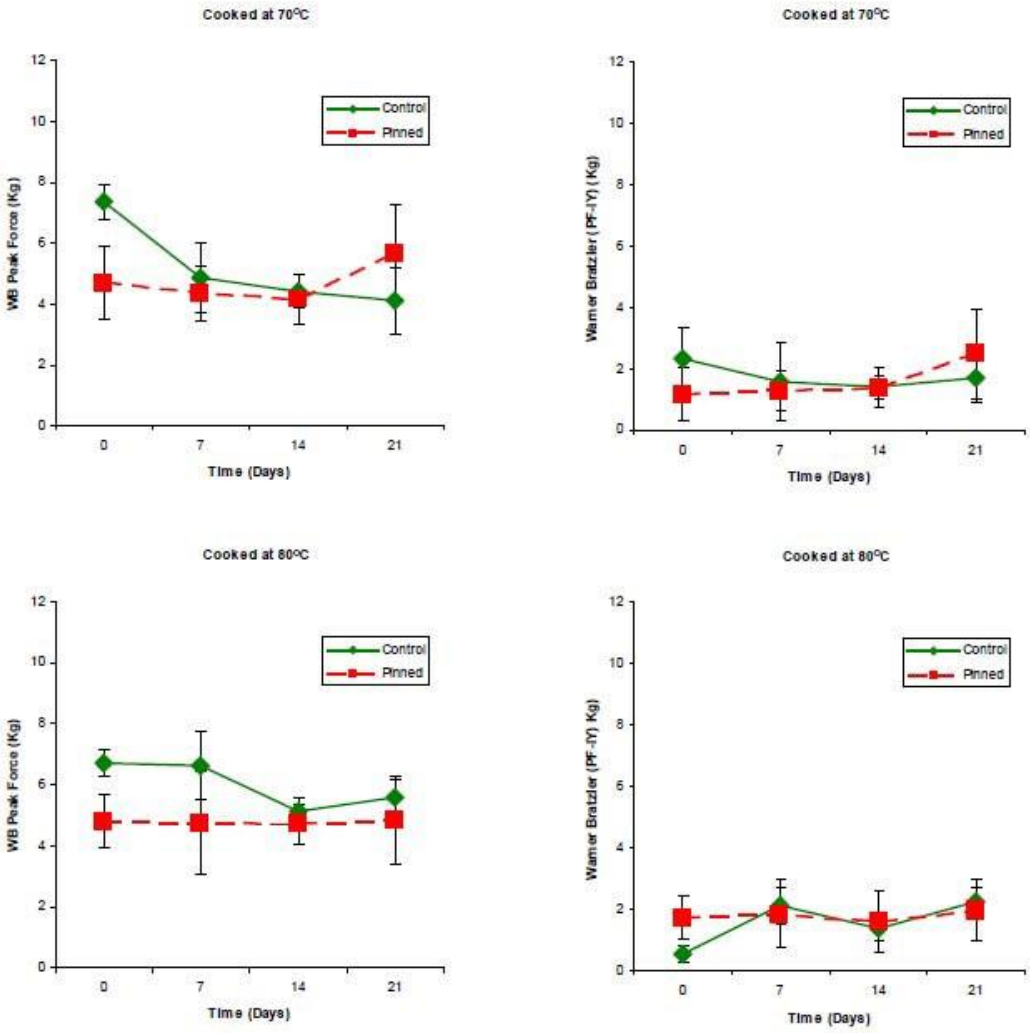


Figure 10: Effect of pinning and ageing on objective texture measurements of cooked *M. biceps femoris*. PF (left) and PF-IY (right) are shown for samples cooked at 70°C (top) or 80°C (bottom)

M. semimembranosus

Control *M. semimembranosus* cooked at 70°C showed no improvement in tenderness with ageing until Day 21. Compared with Controls, pinning resulted in significant reductions in peak shear force only at Day 7 (*P<0.001*) and Day 14 (*P<0.005*) although in general the pinned samples tended to be lower (figure 11). Pinned samples showed a consistent improvement in tenderness over the 21-day ageing period.

When *M. semimembranosus* was aged and then cooked at 80°C there was an incremental improvement in tenderness over the whole storage period. For the Controls, peak shear force values reduced from >9 kg to about 4-5 kg (figure 11). At each time point measured, pinning resulted in a further reduction of peak shear force and this was significant at Days 7, 14 and 21. Although not shown, the improvement in tenderness through both ageing and pinning was through changes in the myofibrillar component as the measured 'initial yield' values paralleled

the peak shear force values. The contribution of connective tissue (calculated from 'peak force minus 'initial yield', PF-IY) was largely unchanged.

Overall, the tenderness of *M. semimembranosus* cooked at 80°C significantly improved with ageing (about 4 kg reduction), and pinning resulted in a further improvement in tenderness of about 1 kg peak shear force. It is evident from the PF-IY data that neither ageing nor pinning had any significant affect of the contribution of connective tissue to overall texture (figure 11).

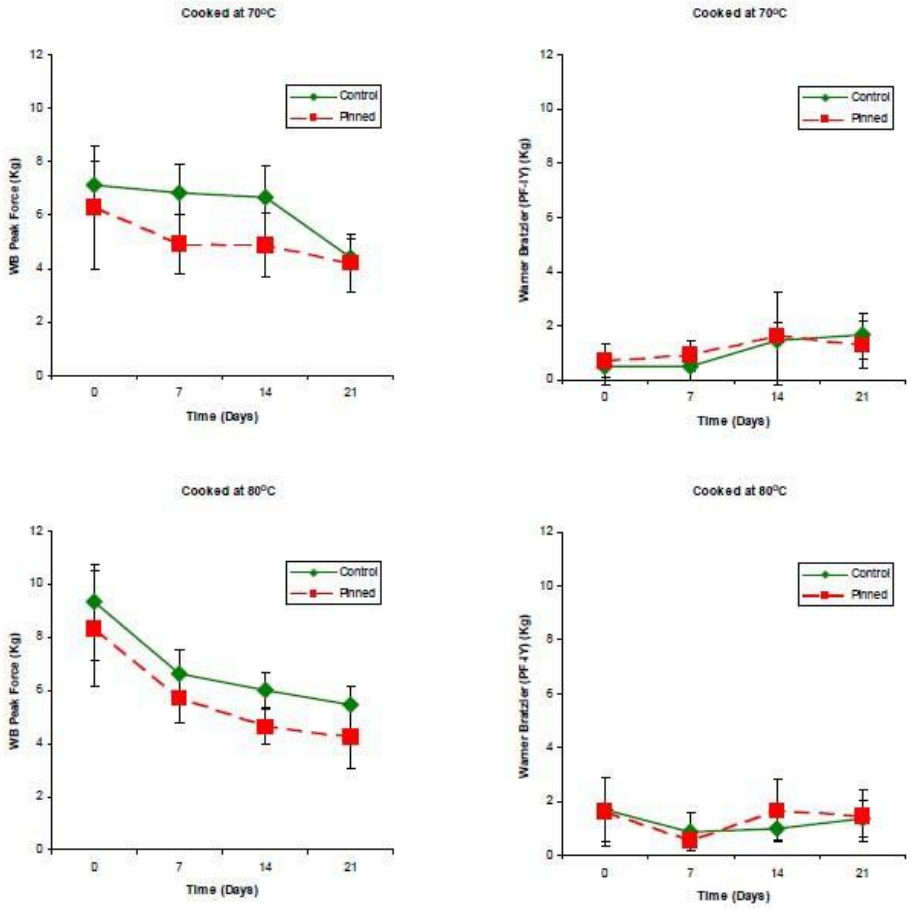


Figure 11: Effect of pinning and ageing on objective texture measurements of cooked *M. semimembranosus*. PF (left) and PF-IY (right) are shown for samples cooked at 70°C (top) or 80°C (bottom)

M. rectus femoris

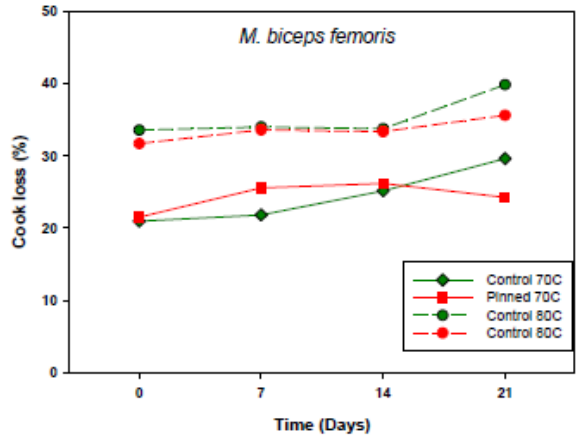
The Control muscles were quite tender, given that peak shear force values were about 4-5 kg. There was essentially no change in peak shear force throughout the 21-day ageing period (figure 12) suggesting that this muscle did not 'age'. This was the case for product cooked at both 70 and 80°C although the 14 day aged muscle cooked at the higher temperature showed in increase in peak shear force. Overall the findings for treatment of *M. rectus femoris* were fairly inconsistent and may have resulted from the variability in the individual samples selected.

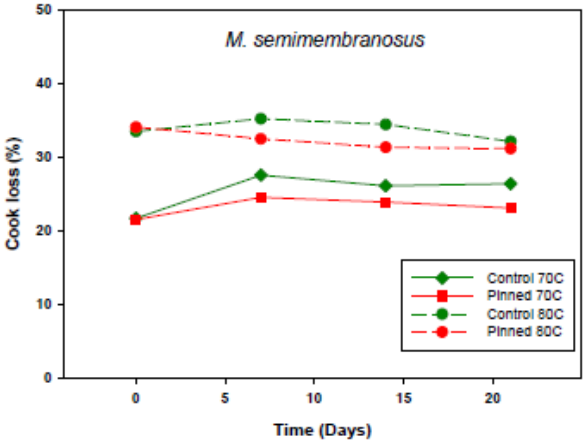
Again it is evident from the PF-IY data that neither ageing nor pinning had any significant effect on the contribution of connective tissue to overall texture (figure 12).

Inexplicably but consistently, the pinned samples, when cooked at either 70 or 80°C, had significantly higher shear values than the controls (*P<0.001*). The pinned muscles however did show an improvement in tenderness with time of ageing unlike the Control muscles. However, the Control muscles had quite low peak shear values on Day 0 and therefore there was not a lot of scope for improvement unlike the pinned muscle that began with a peak shear force of about 9 kg.
Had this muscle not been in rigor when pinned it is possible that a stimulation of contraction may have occurred allowing the muscle to go into rigor in a contracted state. However, this was not the case for meat used in this trial which was at least 24-hours post-mortem and had a pH of about 5.5 - 5.7 when pinned. It is more likely that the findings result from the effects of random sampling of the relatively small pieces of muscle. It is therefore suggested that these findings for pinning of *M. rectus femoris* be treated with caution until this is verified by findings from repeat trials.

3.2.2 Cook loss

Cook losses, expressed as a percentage of original weight, were consistently lower for all muscles when cooked at 70°C compared with those cooked at 80°C (figure 13). Also, cook losses were slightly higher for knuckle compared with the other two muscles. Importantly, pinning did not result in any significant change in cook loss.





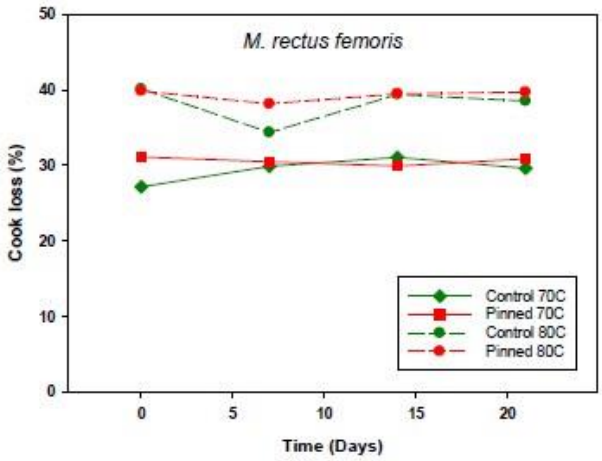


Figure 13: Effect of pinning and ageing on the cook loss from each of the three muscles used (mean values displayed)

4 Discussion

4.1 Meat safety

The above study confirms the hypothesis that blade tenderisation has the potential to transfer bacteria present on the surface of primal cuts to the inside, as has been demonstrated by Johnston *et al.* (1978). Those authors inoculated the surfaces of rounds with Salmonellae, and found those that were blade tenderised had higher numbers of salmonella in core samples than those that were not tenderised. Similarly, Phebus *et al.* (1999), and Sporing (1999) indicated that blade tenderisation transferred 3-4% of surface contamination to the interior of the muscle, regardless of initial surface microbial load. Raccach and Henricksen (1979) believed that there was the potential for a blade tenderiser to become an 'inoculating machine', and recommended strict sanitation of the blades with an iodine-based solution, although they failed to recover more than 1 log cfu/g APC from the centre of tenderised outside rounds which yielded between 1 and 4 log cfu/g APC on the surface. Petersohn *et al.* (1979), on the other hand, failed to demonstrate a significant difference between the APC, psychrotrophic and anaerobic counts of deep tissue samples taken from tenderised and non-tenderised striploin.

In the United States in June 2003, an outbreak of *E. coli* O157 was traced to frozen steaks, sold by door to door vendors, that had been processed first by injection of a 12% solution that included water and flavourings and then by multiple passes through a blade tenderising apparatus (Swanson Laine *et al.* 2005). *E coli* O157 was isolated from the interior of the steaks and it was concluded that blade tenderisation most likely transferred the *E. coli* O157 from the surface. The outbreak was further precipitated by the fact that the steaks were then not properly cooked by consumers, allowing the organism to survive. The authors recommended that the food service industry and public should be aware of the increased risk posed by "non-intact" steaks. Adequate cooking is an important risk mitigation step for tenderised meat. Phebus *et al.* (1999) showed that oven broiling blade-tenderised steaks to an internal temperature of 60°C or higher was effective in ensuring at least a 5 log reduction in *E. coli* O157:H7. Johnston *et al.* (1978), however, found that some Salmonellae survived on the surface and in the core of rounds cooked to an internal temperature of 54.4°C (130°F) and results from Patel *et al.* (2005) also concluded that because the survival of *E. coli* O157:H7 was always higher in blade-tenderised steaks than in untreated steaks cooked to the same temperature, surface bacteria had migrated to the centre

where they were protected from the heat. They found that *E. coli* survived in blade tenderised steaks cooked to 71.1°C (medium done) on an open hearth electric grill.

A USDA risk assessment for tenderised and non-tenderised beef (USDA-FSIS 2002) concluded that the probability of *E. coli* O157:H7 surviving typical cooking procedures is miniscule. They estimated that 3.7 of every 10 million tenderised steaks contain one or more bacteria, compared with 2.6 of 10 million non-tenderised steaks. From literature cited, illness seldom occurs at doses less than 10 bacteria per serving, and at doses of 100 bacteria, approximately 16% of those exposed will become ill. The fraction of tenderised servings with doses ≥100 was estimated to be about 1.5 in 10 million. This means that one illness may be expected per 14.2 million servings. By comparison, for non-tenderised steaks the fraction of servings with ≥100 was estimated to be about 1.4 in 10 million, or one illness per 15.9 million servings.

During storage, the microbial load in deep tissue decreased. This is apparently in contradiction to the results of Boyd *et al.* (1978), who demonstrated an increase in microbial load on *semimembranosus* muscles in the initial two weeks of storage under vacuum. Those authors, however, took samples from ground meat, which would have incorporated surface meat as well as deep tissue, whereas the current study evaluated only deep tissue contamination. The microbial load on the surface would be expected to increase in the initial weeks of vacuum storage, as the Lactic Acid bacteria associated with vacuum-stored meat develop. Petersohn et al. (1979) also demonstrated an increase in internal microbial load during storage under vacuum for 10 days. However, those authors stored the meat as vacuum-packed steaks in a retail-display cabinet at 5°C, whereas the current study stored the meat as vacuum-packed subprimals at 0°C. In general, the degree of tenderisation achieved with blade tenderising increases as the number of passes through the machine increases (Miller 1975). But, increasing the number of treatments may also lead to increased drip and cooking loss (Glover *et al.* 1977), although other authors (Bowling *et al.* 1976) showed no effect on cooking loss. Boyd *et al.* (1978) showed that multiple passes through the pinning machine, up to three times, gave no increase in microbial load of the meat, whereas muscles that had been pinned four times did carry a higher microbial load. This may be as a result of contamination through excessive handling and microbial outgrowth as a result of the length of time removed from the chill store.

4.2 Meat tenderness and cooking loss

In this work we have observed that the individual muscles behaved differently to ageing and pinning. Beef *M. semimembranosus* showed a significant improvement in tenderness as a result of ageing whereas with *M. rectus femoris* ageing showed essentially no effect. Pinning was most effective for *M. semimembranosus* and for *M. biceps femoris* resulting in an improvement in shear value of about 1 kg but the effect was variable in other muscle. However overall, the benefit of pinning was much less than that gained from ageing in those muscles that did respond to ageing. Others have also found the effect of pinning to be variable and dependent upon muscle used (Kolle *et al.* 2004). Although objective measurements of tenderness can detect differences between non-pinned and pinned samples there is evidence that these differences are not always detected by sensory evaluation (Seideman *et al.* 1977, Tatum *et al.* 1978). Therefore these small but significant reductions in shear force may not relate to perceivable improvements in eating quality.

Previous reports have suggested that where pinning or blade tenderisation has been shown to be effective it is believed to be largely through disruption of connective tissue (Glover *et al.* 1977, Seideman *et al.* 1977, Jeremiah *et al.* 1999). However in our studies there was no evidence that connective tissue had been affected. With the muscles used here, irrespective of cooking

temperature, the calculated ‘peak force’ minus ‘initial yield’, which is regarded as a measure of the contribution of connective tissue to meat toughness showed no significant effect with pinning. Where the effect of ageing and pinning was apparent there was evidence from the ‘initial yield’ values that it was changes in the myofibrillar component that was leading to an improvement in tenderness. It is evident from the PF-IY data that neither ageing nor pinning significantly affected the contribution of connective tissue to overall texture.

As expected, cook losses were consistently lower for all muscles when cooked at 70°C compared with those cooked at 80°C and it was also noticed that losses were slightly higher for *M. rectus femoris* compared with the other two muscles. Importantly, pinning did not result in any significant change in cook loss which is consistent with that found by Schilling *et al.* (2003) and George Evins *et al.* (2004), and only a small increase was observed by Obuz and Kropf (2002).

5 Conclusions

Peak shear force was measured to assess tenderness of muscles that had been subjected to pinning treatment together with a period of vacuum-pack, chilled ageing for up to a period of 21 days. The findings were different for each of the muscles investigated. For Control, non-pinned muscles, *M. semimenmbranosus* showed a highly significant improvement in tenderness over the ageing period whereas *M. rectus femoris* was little changed by ageing. There was some evidence of improved tenderness with ageing of *M. biceps femoris* but the effect was not as clear as with *M. semimembranosus*.

Except for *M. biceps femoris*, the other muscles investigated did show an improvement in tenderness, but generally the magnitude was low compared with the effect of ageing.

Pinning did not significantly affect cooking losses when cooked at either 70 or 80°C.

It is evident from the PF-IY data that neither ageing nor pinning had any significant effect on the contribution of connective tissue to overall texture.

Microbiologically, pinning can and does transfer microorganisms from the surface of the primal to the deep tissue. The proportion transferred can range from zero to 63%, but on average it is around 3-5%. If pathogens were present on the surface of the primal, they would be expected to be present at very low levels, so the numbers migrating into deep tissue would be very low. Thus, a USDA risk assessment concluded that the risk of illness from blade tenderised meat is only marginally greater than the risk of illness from non-blade tenderised meat.

6 Further Work

The project proposal originally suggested that the next stage of the project would be to compare the following set of treatments:

1. Control: vacuum pack and store under refrigeration for 21 days. Portion into steaks and simulate retail display under overwrap for 3 days

2. Tenderise immediately, portion into steaks. Simulated retail display under overwrap for 3 days

3. Tenderise immediately, vacuum pack and store for 48 hours to simulate distribution. Portion into steaks and simulate retail display under overwrap for 3 days

4. Tenderise immediately, vacuum pack and store for 7 days. Portion into steaks and simulate retail display under overwrap for 3 days

5. Tenderise immediately, vacuum pack and store for 14 days. Portion into steaks and simulate retail display under overwrap for 3 days

6. Tenderise immediately, vacuum pack and store for 21 days. Portion into steaks and simulate retail display under overwrap for 3 days

7. Vacuum pack and store for 48 hours to simulate distribution. Tenderise, portion into steaks and simulate retail display under overwrap for 3 days

8. Vacuum Pack and store under refrigeration for 7 days. Tenderise, portion into steaks and simulate retail display under overwrap for 3 days

9. Vacuum pack and store under refrigeration for 14 days. Tenderise, portion into steaks and simulate retail display under overwrap for 3 days

In light of the findings from the work reported here, showing that, particularly for *M. semimembranosus*, after ageing for three weeks the tenderness improvements produced by pinning are minimal; that pinning produces good improvements in tenderness in product that is evaluated in the first two weeks of storage prior to pinning; and that some muscles, in this case *M. rectus femoris*, respond in an unexpected fashion to pinning, it is suggested that treatments 6, 5 and 9 above may be redundant. It would be useful to consider multiple pass pinning, as much of the literature indicates significant improvements in tenderness following double or triple pinning, and also to further investigate the counterintuitive results found with *M. rectus femoris*. It is suggested that treatments 6, 5 and 9 above are replaced with:

A. Tenderise twice immediately, portion into steaks. Simulated retail display under overwrap for 3 days

B. Tenderise twice immediately, vacuum pack and store for 48 hours to simulate distribution. Portion into steaks and simulate retail display under overwrap for 3 days

C. Tenderise twice immediately, vacuum pack and store for 7 days. Portion into steaks and simulate retail display under overwrap for 3 days

All treatments would be carried out using both of *M. semimembranosus* and *M. rectus femoris*, to allow comparison of the two muscles.

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