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Enhancing the Tenderness of Beef

The National Food Centre

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Contents

ENHANCING THE TENDERNESS OF BEEF

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SUMMARY

This project investigated various methods which had potential to increase beef tenderness and was also aimed at elucidating the biochemical mechanism underlying the improved tenderness.

Post slaughter manipulation of carcasses greatly reduced the variability in tenderness between carcasses and also enhanced the inherent tenderness.

Temperature at which a carcass was held before it entered rigor greatly affected its tenderness. Holding carcasses for 10 hours at either 6, 8 or 10°C after slaughter resulted in different degrees of tenderness. The lower the temperature the greater the risk of cold-shortening. Furthermore, lower temperatures reduced the degree of meat protein breakdown.

pH is very important in the tenderisation process. If meat has a very high final pH (above 6.3) there is evidence that calpains (enzymes responsible for much of the protein breakdown which occurs during ageing) are most active resulting in more tender meat. Intermediate final pH (5.8 - 6.3) produced meat tougher than normal. This project revealed also that carcasses with fast rates of pH fall produced more tender beef than slow rates of pH fall. Carcasses with slow rates of pH fall had shorter sarcomeres and less degree of proteolysis.

Carcasses hung by the aitch bone (hip) were more tender than those hung conventionally by the achilles tendon (hock). Electrical stimulation (low voltage) was useful if carcasses have a potential to cold-shorten during chilling (e.g. slow glycolysing muscles or lean carcasses). Double electrical stimulation had no effect.

Despite popular opinion no major differences were noted in the quality of beef aged on the bone or vacuum packed.

Other methods examined include calcium-ion injection and high pressure technology.

In collaboration with partners at the University of Athens and The University of Tübingen, isolation and identification of protein fragments was carried out. Not a great deal of proteolysis occurred despite the improvement in tenderness during ageing. It is concluded that the small degree of proteolysis during ageing occurred on highly specific and structurally important regions of the muscle fibre. C-protein may be one of these sites.

INTRODUCTION

The importance of providing end users with a food product of consistent quality has never been greater. Long gone are the days when meat was sold on the basis of its weight, leanness and "smell". It is not surprising then, that there has been a resurgence of research and development in the form of scientific publications, stringent specifications from supermarkets to their suppliers, consumer appraisal and knowledge coupled with sustained marketing techniques. The need within the meat industry for consistently high quality beef continues to grow in importance because of the pressure from a variety of sources such as health authorities, national quality schemes, large supermarket chains, labelling and marketing requirements and the need to satisfy the more discerning consumers.

Despite the importance for the above, meat remains a highly variable food in terms of quality. The reasons for its variability are many, such as different animal production systems. However, today's meat industry is supplied with material from a variety of sources. This limits the control which the meat processor has over variability at the pre-slaughter stage.

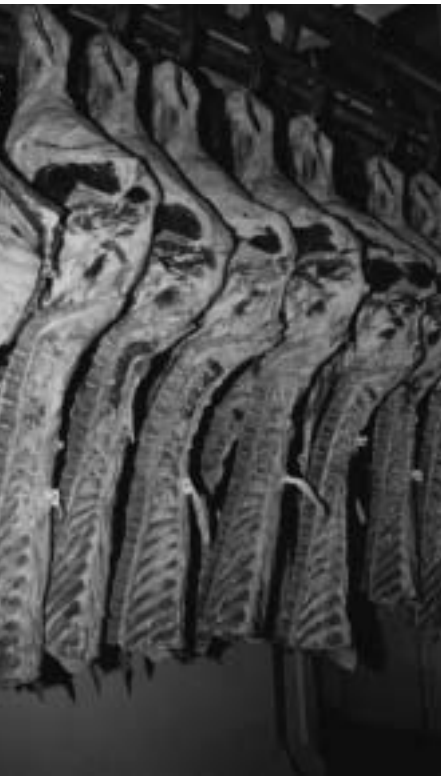
Control of quality during processing, especially early post-mortem, is possible within limitations. Many methods of enhancing quality are fast emerging. Some of these need to be adopted and modified by the meat industry, while others have yet to be scientifically verified. This project aims to evaluate some emerging technologies and attempts to understand the biochemistry underlying their effects on meat tenderness.

Meat quality

A common definition of quality is that it is a "measure of traits that are sought and valued by the consumer". The most technically useful definition with respect to meat was described by Hoffman (1987) who stated that meat quality is "the sum of all quality factors of meat in terms of the *sensoric, nutritive, hygienic, toxicological and technological* properties".

Sensory properties include tenderness, colour, flavour, odour and juiciness while nutritive factors include fat and protein content as well as vitamins,

minerals and biological value. Hygienic and toxicological factors include spores, moulds, bacteria, toxins and residues, and finally technological factors include water-holding capacity, pH, water distribution etc. In meat processing, the question arises how can we control and improve these properties. Hygienic and toxicological properties are mainly extrinsic and can be controlled by HACCP and good manufacturing procedures to reduce risks, and by constant monitoring. However, the other factors are intrinsic and are determined by pre- and post-slaughter influences. Some of these properties can be improved by technological handling of the carcass, others can only be monitored and the meat selected for particular needs, others presently can only be measured after cooking at which time it is too late to rectify if required. There would be little pressure or requirement to monitor, evaluate, control or improve these properties if meat were not one of the most inconsistent and diverse foods. Because beef quality is so variable, there is a particular need to find ways to measure it soon after slaughter, so that steps to improve it can be taken.



Post-slaughter manipulation of carcasses can produce more tender beef

Variability of beef quality

The variability of meat quality stems from many sources (Troy, 1995). Pre-slaughter factors include breed, sex, age at slaughter, feed, handling and environment, type of muscle and carcass composition. These factors affect the sensoric, nutritive and technological attributes of the finished product. Meat processors often have little control over these factors, but need to take into account our current knowledge of quality differences emanating from such factors. Furthermore, the post-mortem handling of carcasses has great influence on meat quality. The critical time for this to happen is during rigor onset, before the muscle goes into rigor mortis.

Slaughter and rigor development

The biochemical events in muscle after death are well documented. Due to the oxygen supply being depleted after exsanguination, energy metabolism is shifted to the anaerobic pathway, lactic acid is produced and accumulates in the muscle tissues until nearly all the glycogen (energy store) is depleted or until the pH fall inactivates the enzymes of glycolysis (see Hedrick et al., 1994). The rate and extent of the pH fall has profound effects on meat quality.

Attainment of low pH in a "warm" environment (slowly chilled carcasses) causes denaturation of muscle proteins. Denaturation causes loss of protein solubility, loss of water holding capacity and reduced pigment colour intensity. Hence muscles with very rapid pH decline will be pale, soft and exudative (PSE). Conversely muscles which maintain a high pH (due to lack of lactic acid production caused by reduced energy stores after slaughter) are dark, firm and dry (DFD).

The other major significant phenomenon related to early post-mortem events is the development of rigor mortis. As ATP is depleted, permanent actomyosin cross-bridges form, causing the muscle to contract resulting in shorter sarcomeres. The muscle becomes more rigid. However, during storage at refrigeration temperatures after the onset of rigor, many changes occur which alter meat quality. The most notable changes relate to the degradation of key structural myofibrillar proteins, and this period is termed conditioning or ageing of the meat.

The normal practice for many years to prevent "cold shortening" is to hold the carcass above 10°C for 10 hours immediately after slaughter (the 10/10 rule). In this way severe contraction of the muscle fibres is prevented. If severe contraction is allowed to occur by subjecting the carcass to a lower temperature early post-mortem the beef, despite the ageing process, will remain extremely tough to eat. However, carcasses and individual muscles can vary in their response to this phenomenon. For instance the outer muscles such as the striploin will be subjected to a faster fall in temperature during chilling than the internal muscles such as the silverside. So this guideline is not definitive. Furthermore, a lower temperature reduces the activity of natural tenderising enzymes which become activated immediately after death.

General Methods

Most work was done using Hereford cross heifers, 20-24 months old. Animals were slaughtered (captive bolt stunned and exsanguinated conventionally) at The National Food Centre or in Meadow Meats Ltd., Rathdowney, Co Laois. Excised striploins (M. *longissimus dorsi*) or other muscles (where stated) were aged under control conditions of 0° to -2°C up to 14 days post-slaughter generally.

Tenderness measurements were taken by standard protocols. Shear force was measured using an Instron Model No. 1140 with a Warner-Bratzler blade attachment. Sensory analysis was carried out using the protocols of the American Meat Science Association (AMSA, 1978). Steaks were grilled to an internal temperature of 70°C and given to trained taste panellists. Panellists were asked to assess the samples on a hedonic scale of 1 to 8 as follows:

Tenderness -	scale 1-8;	1 = extremely tough,	8 = extremely tender
Juiciness -	scale 1-8;	1 = extremely dry,	8 = extremely juicy
Flavour -	scale 1-5;	1 = very poor,	5 = very good
Overall acceptability -	scale 1-6;	1 = not acceptable,	6 = extremely acceptable.

Other methods can be found in O'Halloran et al., (1997).

The effect of different chilling regimes on the tenderness of beef steaks

Twelve Hereford cross and 12 Charolais cross steers were selected and assigned to three different chilling regimes. Carcasses were placed at 1 h post-mortem into a chill at 10°C, 8°C or 6°C ambient for 10 hours after which time the remainder of the chilling regime was normalised at 0°C to 2°C ambient.

Results showed that initial chilling at 6°C caused a noticeable decrease in tenderness compared to the 8°C and 10°C chilling regimes (Table 1). Shear force values were higher and the meat was tougher to eat after 2 and 7 days ageing.

Differences were greater at the early stages of ageing, indicating that the chilling regime affects the rate of ageing. Even at 14 days of ageing the 6°C chilled meat was about 10% tougher than the 10°C chilled beef. Further analysis revealed that the cause of this increased toughness was the reduced ability of the muscle enzymes to break down structural proteins at the lower temperature.

In conclusion, it is important that carcasses are held at about 10 to 13°C for the first 10 h post-mortem to allow the full potential of the tenderising enzymes to be achieved.

Table 1: Effect of early chilling temperature on the tenderness of beef striploins after 7 days ageing

Chilling temperature	10°C	8°C	6°C
Shear force (kg)	3.93	4.25	5.71
Tenderness	5.0	6.0	5.7
Overall acceptability	3.6	4.1	4.0

It was suggested by May et al., (1992) that the internal striploin temperature (4.5 cm to 5.5 cm into the medial portion of the *longissimus dorsi* immediately anterior to the 9th rib) at 2.5 h post-mortem was closely related to the tenderness of the meat. If this is so, perhaps closer monitoring of pH and temperature should be investigated throughout the carcass. Certainly using the 10/10 rule, most cold toughening can be avoided. However, temperature and pH distribution vary throughout the carcass.

Carcass chilling temperature is very important for tender meat

The effect of the final pH on meat quality

The influence of the ultimate pH (pH_u measured at 48 h post-mortem) on the sensory quality of beef was assessed.

Three groups of four Hereford cross Friesian heifers were used in this study. Two groups were given a pre-slaughter, subcutaneous injection of adrenaline to produce muscles with high (6.3 - 6.9) and medium (5.8 - 6.2) pH_u values. The muscles from the control group had a normal pH_u of 5.4 to 5.7.

The high pH_u carcasses produced the most tender meat as measured by sensory analysis and shear force measurements (Table 2). High pH_u meat (striploin) scored on average 6.9 on a 1 to 8 scale for tenderness compared to 5.3 and 5.5 for intermediate pH_u and control meat, respectively, after 14 days ageing. Shear force values agreed with these results i.e. the least force (2.98 kg per 1.25 cm core) was required to shear muscle from the high pH_u meat compared to control meat (4.20 kg per 1.25 cm) after 14 days ageing.

Table 2: Quality of beef striploins from carcasses with different final pH values after 7 days ageing

Ultimate pH range	5.4 - 5.7	5.8 - 6.2	6.3 - 6.9
Shear force (kg)	6.26	7.14	3.35
Tenderness	4.3	4.6	6.6
Overall acceptability	3.5	3.6	4.3

A greater effect was observed at the earlier times post mortem (day 2 and 7). High pH_u meat was not only observed to be more tender but also more juicy. Studies of sarcomere lengths, protein degradation and enzyme activity revealed that high pH_u meat underwent a higher level of myofibrillar breakdown, had a higher level of proteolytic activity and its sarcomere length was not different.

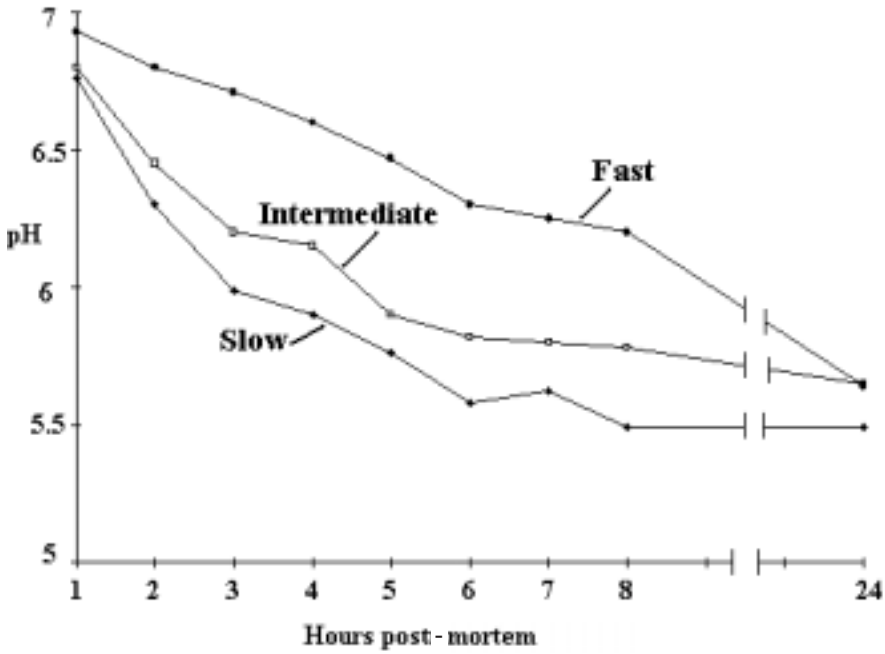
In conclusion, the ultimate pH of meat plays an important role in its final tenderness. Very high pH meat is more tender than control but intermediate pH_u meat can be tougher than control. Similar trends were noted for the topside cut but to a lesser extent.

The relationship between early post-mortem pH and beef tenderness

The rate of early post-mortem pH fall in beef striploin was examined to determine its influence on the rate and extent of the tenderisation process. The pH of striploins from Hereford cross heifers was taken for 24 hours post mortem. Twenty four striploins were then selected according to their rate of pH fall: eight slow, eight intermediate and eight fast, Fig. 1. The muscles were allowed to age normally over a 14 day period. Samples were taken for tenderness measurements at 2, 7 and 14 days.

Tenderness was greatest in the striploins with fast falling pH (Table 3). These muscles were significantly more tender at 2 and 7 days post mortem. Shear force values were also significantly different at 14 days storage. Sensory analysis confirmed that muscles with faster falling pH are more tender at 2 and 7 days post mortem.

▼ Figure. 1:The post-mortem pH decline of slow, intermediate and fast glycolysing beef striploins (M. *longissimus dorsi)* during the first 24 h post-mortem



Analysis of the sarcomere length indicated that muscle shortening was not a major factor in the differences in texture. Although some shortening was noted at 2 days post mortem in the striploins with slow falling pH, most evidence points to the increased breakdown of myofibrillar proteins coupled with enhanced activity of proteolytic enzymes in the fast falling pH muscles, as the factors causing tenderness.

In conclusion, the results show that early post-mortem pH plays an important role in the tenderisation process. Shortening can occur more readily in slow falling pH muscle but the major contribution to the increased tenderness is through the effect of pH on the endogenous enzyme systems responsible for muscle protein breakdown.

As variability in the rate of pH decline between carcasses may be very high, this may partly explain the inconsistency in meat tenderness in beef from different animals.

Measuring meat tenderness using a Warner Bratzler device attached to an Instron Universal Testing Machine

Table 3: Quality attributes of striploins (M. *longissimus dorsi*) from beef carcasses with different rates of pH decline after slaughter

pH decline		Fast	Intermediate	Slow
Tenderness (sensory panel) :				
Ageing time	2 days	4.2	4.1	3.2
	7 days	6.2	5.7	4.2
Overall acceptability:				
Ageing time	2 days	3.3	3.2	2.7
	7 days	4.3	4.2	3.3
Shear force (kg)				
	2 days	3.43	4.24	6.3
	7 days	2.62	3.02	4.72

Aitch bone hanging

The effect of aitch bone hanging (or pelvic suspension) on the tenderness of beef striploins was examined.

Conventional (left) and aitch bone (right) methods of hanging carcasses

Carcasses from 12 heifers were used. One side of each carcass was hung by the aitch bone (AB) after final washing and just before entering the chill. The paired side was hung by the conventional method. After 48 h storage at 2°C, four cuts were excised from each side. They were the striploin (*longissimus dorsi*), topside (*semimembranosus*), silverside (*biceps femoris*) and rump (*gluteus medius*). Each muscle was then sampled at 2, 7 and 14 days of storage for sensory analysis, sarcomere length, shear force and cooking loss. (Table 4)

The sarcomere length, a measure of muscle extension or stretch, was increased considerably in AB hung muscles. The average increases were 15%, 30%, 33% and 30% in the striploin, topside, silverside and rump, respectively. Shear force was lower in steaks from AB carcasses especially in the striploin and topside and to a lesser extent in the silverside and rump. The latter muscles contained more collagen component which may mask some of the advantages of altering the myofibrillar component of toughness. However, after ageing for 14 days the striploin muscle from conventionally hung animals had shear force similar to AB carcasses.

Table 4:Effect of carcass hanging method on the tenderness of beef hindquarter cuts

Muscle	Conventionally hung	Aitch bone hung
Striploin		
Sarcomere length (µ)	1.71	2.04
*Shear force (kg)	4.50	3.87
*Tenderness	5.6	6.2
*Overall acceptability	3.9	4.2
Topside		
Sarcomere length (µ)	1.69	2.43
*Shear force (kg)	6.26	5.12
*Tenderness	3.6	5.2
*Overall acceptability	2.9	3.7
Silverside		
Sarcomere length (µ)	1.79	2.68
*Shear force (kg)	5.83	5.30
*Tenderness	3.5	5.0
*Overall acceptability	3.0	3.6
Rump		
Sarcomere length (µ)	1.78	2.51
*Shear force (kg)	4.93	3.92
*Tenderness	4.9	5.9
*Overall acceptability	3.2	4.1

* After 7 days ageing µ=microns

Sensory analysis showed that panellists consistently rated all the AB muscles at all times post-mortem more tender than conventionally hung muscles. Greatest effects were seen at the early stages of conditioning (day 2). Trained panellists rated the AB steaks on average 20% more acceptable. AB samples were found to be less chewy and less firm. No increase in cook loss was detected. Protein profiles of the muscles showed no difference in proteolytic patterns between AB and conventionally hung beef.

In conclusion, aitch bone hanging of carcasses improves the tenderness of most of the important commercial cuts. Its effect is greatest early post mortem. It does not affect cook loss nor does it exert its action through enzymatic pathways.

Electrical stimulation

Electrical stimulation (ES) of beef carcasses has been a common practice for many years. Briefly, it increases the rate of post-mortem glycolysis resulting in a rapid pH decline. This decline is accompanied by higher post-mortem temperatures, increase in drip loss in most cases and brighter colour associated with the higher light reflection of meat. ES also induces rigor early post-mortem, hence reducing the risk of cold-shortening if the carcass is chilled rapidly. The latter implies a tenderisation effect if cold-shortening is possible.

There is some evidence for three other mechanisms of tenderness brought about by ES: (1) disruption of the lysosomal sac with subsequent release of cathepsins (proteases) at low pH/high temperature environment; (2) physical disruption of the muscle fibres, especially with high frequency electrical stimulation; (3) reduction in collagen cross-linking. However, if these three modifications were induced by ES, major and consistent benefits to meat tenderisation would be inevitable. This is not the case and some reports suggest that stimulation even induces a toughening effect by the deactivation of important natural enzymes early post-mortem. The reason for the many conflicts is possibly due to the variability of the process of ES, (frequency, pulse duration, voltage, mode and time of application), the type of animal, the chilling rates and the location of the muscle examined. Previous work had shown that low voltage ES did not increase substantially the tenderness of beef steaks compared to non-stimulated muscles, when cold-shortening was not possible.

The effect of double electrical stimulation on the tenderness of beef striploin (M. *longissimus dorsi*)

Recently the application of ES at 10 min and again at 40 min post-mortem has become part of some retail specifications. This study evaluated the effects of double electrical stimulation (DES). Eight Hereford-cross heifers were slaughtered conventionally. Four carcasses were stimulated once, split and one side of each was stimulated a second time. Four non-stimulated (NES) carcasses served as controls. In all cases the ES carcasses showed a faster drop in pH than controls. There was little difference in rate of pH fall between single ES and DES. The striploin (M. *longissimus dorsi*) was excised after 48 h and sampled at days 2, 7 and 14 post-mortem (4°C storage) for assessment of proteolysis by electrophoresis (SDS-PAGE), sarcomere length measurement, mechanical shear force (Warner Bratzler) and sensory analysis.

In all cases, progressive tenderisation was detected over the ageing period. The pattern of proteolysis was the same for all samples, although a 30 kDa band was more pronounced after 2 days ageing in samples from ES and DES carcasses than in the controls. The mean sarcomere lengths of all the samples were similar (1.73mm). There were no differences in sarcomere length between ES and DES treatments. Sensory analysis showed that panellists did not detect any differences in tenderness between NES, ES and DES (Table 5). Warner-Bratzler shear force values confirmed these results. It is concluded that DES has no positive effects on sensory attributes.

Table 5: Tenderness of striploins from control (NES), stimulated (ES) and double stimulated (DES) beef carcasses after 14 days ageing

	NES	ES	DES
Shear force (kg)	4.93	5.15	5.10
Tenderness	5.7	5.7	5.5
Overall acceptability	3.6	3.6	3.7

The effect of ageing methods on the tenderness of beef

Despite the common belief that ageing beef "on the bone" increases the eating quality, in terms of flavour and tenderness, compared to vacuum packed meat, few scientific studies have been carried out. Because of the general consumer-led pressure to produce food products in a more traditional manner, ageing on-the-bone has recently become part of some specifications. The objective of this work was to compare the eating quality of beef aged on the bone and beef aged under vacuum over a period of 28 days.

Eight Hereford cross heifers were slaughtered conventionally. Alternate sides were assigned to "on the bone" (OB) and vacuum packaging (VP) treatments. Striploins (M. *longissimus dorsi*) were excised 48 h post-mortem from one side of each carcass for the vacuum packaging treatment. The striploin of the other side was aged on the carcass. The vacuum packed cuts were stored along with the hind-quarters at 0°C throughout the ageing period of 28 days. Samples were taken for protein electrophoresis and sarcomere length, and steaks (2.5 cm) were taken for sensory analysis assessment and mechanical measurements.

Electrophoresis profiles of extracted myofibrillar proteins showed little variation in proteolysis between treatments. The 30 kDa band appeared at the same time in both ageing regimes as well as the loss of the 55 kDa band corresponding to desmin. The VP ageing method resulted in increased intensities of the 22, 27 and 30 kDa bands.

An eight member in-house taste panel scored steaks cooked to 70°C for tenderness, juiciness, overall flavour and overall acceptability (Table 6). After 7, 14 and 21 days ageing the VP treatment had higher tenderness scores than the OB samples. These differences were significant ($P<0.01$) at 14 days only. On extended ageing of samples to 28 days there was no difference between OB or VP treatments. Improved juiciness scores were recorded on each day of ageing except day 2 for the VP treatment; however these differences were not significant ($P>0.05$). Ageing on the bone is perceived to be a more traditional method of ageing by the consumer and to have beneficial effects on flavour. Flavour attributes were unaffected by ageing method or by length of time of ageing, as judged by panellists in this study.

Table 6: Eating quality of beef striploins (M. *longissimus dorsi*) aged "on the bone" (OB) or in vacuum packs (VP)

Days of ageing	2		7		14		21		28	
	OB	VP	OB	VP	OB	VP	OB	VP	OB	VP
Shear force (kg)	8.25	7.64	6.09	5.72	4.46	4.72	5.25	4.16	4.06	4.11
Tenderness	3.4	3.3	5.1	5.40	5.4	6.1	5.4	5.6	6.1	6.1
Juiciness	4.5	4.2	5.1	5.3	4.1	4.6	4.4	4.4	4.6	4.9
Flavour	3.5	3.5	3.8	3.7	3.6	3.8	3.6	3.3	3.6	3.6
Overall acceptability	2.8	2.6	3.7	3.9	3.6	4.1	3.8	3.8	4.0	3.8

Warner-Bratzler results indicated a progressive reduction in shear force values over the ageing period. After ageing to 28 days there was no difference in shear force values ($P>0.05$) between treatments.

In conclusion, firm evidence of differences in tenderness or indeed flavour between "on the bone" or vacuum packed, aged beef was not identified. The eating quality of beef from both methods of ageing was quite similar.

The effect of calcium chloride injection on beef tenderness

In the last eight years there has been a growing interest in improving meat tenderness though the use of calcium chloride ($CaCl_2$) infusion, or injection. It was reported that pre-rigor injection of beef muscles or infusion though the carotid artery of sheep carcasses before evisceration resulted in rapid tenderisation. In fact, maximum tenderisation could occur after only 24 hours storage, when 10% by weight of a 300 mM solution of $CaCl_2$ is injected pre-rigor. Up to 40% increase in tenderness was achieved by workers in the USA. The rationale for $CaCl_2$ injection derived from the hypothesis that it could be possible to activate the calcium-dependent proteases (calpains) thought to be responsible for post-mortem tenderisation. Indeed, $CaCl_2$ injection does increase the degradation of myofibrillar proteins which are also susceptible to calpain proteolysis and accelerates the loss of calpain activity.

Two studies were undertaken during the course of this project.

(a) Pre- and post-rigor injection of $CaCl_2$

Striploins from carcasses of four Hereford cross heifers were excised within 1 h post-mortem and cut into steaks either injected with 300 mM (10% v/w) or 100mM (10% v/w) CaCl2 . Non-injected steaks were used as controls. Post-rigor steaks were excised from similar animals at 21 h post-mortem and treated similarly.

Steaks were aged for 14 days at 4°C . pH, sarcomere length, protein electrophoresis (SDS-PAGE), sensory analysis, shear force, cook loss and drip loss measurements were carried out. Results showed that pre-rigor injection of calcium resulted in rapid onset of rigor. The effect was greatest when the calcium injection was given very soon after slaughter, when the striploin pH was at 6.9. This rapid onset of rigor was coupled with a severe contraction of the sarcomeres often up to 35% shorter than non-injected control steaks.

Pre-rigor injection also accelerated the appearance of the 30 kDa protein breakdown fragment in electrophoresis gels indicating a greater degree of proteolysis. Up to 20% drip loss was measured in samples treated with calcium early post-mortem. Warner-Bratzler shear force of the cooked samples indicated that there was a reduction in force required to shear the calcium treated meat compared with controls during the early stages of storage (2 & 7 days) but this was not the case for fully aged meat (14 days).

Taste panel evaluation indicated that pre-rigor injection of calcium gave more tender meat especially after 2 and 7 days ageing and that there were no adverse flavour effects.

In contrast, post-rigor injection of calcium at the same concentration did not induce the same degree of shortening as in the pre-rigor situation. The 30 kDa protein breakdown fragment appeared more intensely by day 2 compared to controls. It did not appear as strongly as in the case of pre-rigor calcium injected muscle. Drip loss was not as great as for the pre-rigor injected samples but was greater than controls. Shear force values of injected post-rigor samples did not differ significantly from the controls.

It is concluded that pre- and post-rigor injection of $CaCl_2$ accelerates the post-mortem tenderisation process. The higher concentration of calcium has a more marked effect. Calcium did not enhance the overall tenderness of the muscle by 14 days of ageing.

(b) Calcium treated beef: an industrial trial

The objective was to determine, under industrial conditions, the effect of different injections of $CaCl_2$ on the biochemical, physico-chemical, mechanical and sensory characteristics of striploin (M. *longissimus dorsi*) and topside (M. *semimembranosus*). The effects of post-rigor injection of 200 mM and 100 mM $CaCl_2$ into four striploins (10 to 13% w/w) and topsides (9 to 10% w/w) were examined. Muscles were injected about 27 h post-mortem using a Fomaco 40-needle automatic poultry injector. Muscles were then vacuum packed and aged at 4°C. Samples were taken at 2, 7 and 14 days post-mortem and assessed (pH, sarcomere length, electrophoresis, sensory analysis, shear force, texture, initial colour stability and microbial growth).

Although most panellists rated calcium injected beef similar to non-injected beef, some consistently noted a slight bitter and metallic flavour during the early storage period (up to 7 days). Panellists also rated texture more favourable for calcium injected steaks. There was a greater acceleration of the tenderisation process. Calcium treated beef was about as tender after 2 days of ageing as non-injected beef after 7 days ageing (Table 7). However, although higher tenderness scores were given by panellists after 14 days ageing, they were not significant in practice. Spoilage (psychrotrophic) microbial counts (after incubating at 25°C for 72 h) and initial colour stability values of lightness, hue and chroma (after allowing the cut steak to bloom for 5 h) were unaffected by the presence or concentration of CaCl2 . In conclusion, CaCl2 injection gave more tender beef and showed no adverse effect on quality traits.

Table 7: The effect of calcium chloride injection of cuts on the eating quality of beef striploin and topside

Attributes	Days ageing	Control	200 mM $CaCl_2$	100 mM $CaCl_2$
Tenderness	2	4.1	5.5	5.2
	7	5.1	6.2	5.7
	14	5.6	6.0	5.9
Juiciness	2	5.3	5.7	4.8
	7	5.9	5.9	5.6
	14	5.2	5.4	4.9
Flavour	2	3.6	3.5	3.6
	7	4.1	3.9	3.8
	14	4.0	4.0	3.8

It is concluded that $CaCl_2$ injection is useful for meat which is required to be aged rapidly. $CaCl_2$ injection tenderises meat more rapidly but prolonged ageing diminishes its effects significantly.

Summary of other methods of tenderisation of beef evaluated during the course of this Project

High pressure treatment

High pressure treatment to preserve and process foods has been the subject of renewed interest due to developments in the engineering of high pressure equipment. Japan is the leader in this field, and markets high pressure processed foods. Most studies on high pressure treatment of foods deal with bacterial safety aspects.

In recent years workers have subjected meat to high pressure (about 150 MPa or 21,700 pounds per sq inch) for 2 minutes. The results were dependent on the rigor state of meat. Greater reduction of shear force occurred with pre-rigor meat. The mechanism of tenderisation is unclear but is thought to be enzymatic. Pressure induced proteolytic activity was increased by disruption of the lysosome and subsequent release of cathepsins as well as inactivation of the calpain protease inhibitor, calpastatin. Whatever the mechanism of tenderisation, pre-rigor treatment involves time constraints if implemented at industry level. Hence, post-rigor application would be more beneficial to the industry.

The effects of high pressure on post-rigor meat was carried out on small samples. Pressure was applied at 30 MPa. There was evidence of greater proteolytic activity by the rapid and enhanced appearance of the 30 kDa band; however, some of these effects were masked by the denaturation of sarcoplasmic proteins brought about by the high pressure. Despite this no major differences in texture or sensory quality were detected. The colour of the treated meat was severely affected. The lightness and yellowness (L and b values) were significantly increased but the colour of the cooked meat was not affected. This change in colour is due to the oxidation of myoglobin to metmyoglobin and the shift towards lighter tones is caused by coagulation of proteins.

This short trial demonstrated that proteins of meat are modified by application of high pressure but that benefits in terms of tenderness were not evident.

Calcium lactate treatment

Pre-rigor injections of calcium lactate (CaL) led to some similar effects to those obtained for pre-rigor calcium chloride injections: acceleration of rigor onset, large increase in total drip losses; contracted appearance; and less juiciness in sensory assessment compared to controls. Moreover, the problems in flavour perceived in calcium chloride treated samples (bitterness and abnormal flavour) were also observed but with a dose effect. The 300 mM CaL-treated samples were perceived more bitter and with more abnormal flavour than the 100 mM CaL-treated samples. This dose effect (100 mM or 300 mM CaL) was more marked for CaL than for $CaCl_2$ treatment.

Post-rigor injections of CaL were as efficient as post-rigor $CaCl_2$ treatments in accelerating the ageing process and in decreasing the strength of cooked meat. The slight increase of total drip losses and of losses in dry matter was quite similar to that obtained for post-rigor $CaCl_2$-treated samples. Post-rigor injected samples were less contracted, more tender, more juicy and less fibrous than the control from day 2. However, these beneficial effects tended to decrease with ageing time and final tenderness (day 14) was significantly improved only for the higher dose of CaL. Moreover, and in contrast with $CaCl_2$-treated samples at 100 mM, the corresponding injection of CaL (100 mM) did not induce drawback effects on meat flavour.

In conclusion, injection after rigor onset of 10% w/v 100mM CaL led to the best results as the rate of tenderisation of meat was increased without the appearance of defaults in flavour compared to the control. However, after complete ageing, differences in tenderness with the control tended to become non-significant.

Identification of protein fragments in aged beef

The partners in this project undertook work of a fundamental nature in order to underpin the science of the tenderisation process.

The University of Athens (UA), and the University of Tubingen (UT) in collaboration with The National Food Centre identified the proteins which were degraded during the tenderisation process. A summary of the findings is set out below:

Muscle proteins were extracted from carcasses during the ageing process and subjected to electrophoresis techniques. A number of fragments were isolated according to their molecular weight. Their amino acid sequence was partially identified. With this knowledge the parent molecule was identified from data banks. The main protein fragments were 30, 32, 16, 90 and 110 kDalton molecular weights.

Sequence of the 30 kDa fragments. This fragment was confirmed as originating from troponin-T in both laboratories at UA and UT. Sequences of up to 20 amino acid residues long were obtained. As the sequence of bovine skeletal muscle troponin-T is still unknown similarity matches were performed against rabbit and Japanese quail. Results showed between 75-80% similarity match with rabbit skeletal muscle troponin-T and Japanese quail embryonic skeletal muscle troponin.

Isolating protein fragments by electrophoresis

Sequence of the 32 kDa protein fragment. This sequence had a 90% match with human skeletal muscle troponin-T. A common parameter noted between this fragment and the 30 kDa is that the first residue of both fragments is Glu, indicating that the muscle protease responsible for the cleavage of both fragments might be a Glu-N specific one. This is of interest in elucidating the proteolytic mechanisms of ageing meat.

Sequence of the 16 kDa fragment. The sequences obtained from this fragment at first proved elusive. However, both UA and UT separately suggested its origin to be glyceraldehyde-3-phosphate dehydrogenase, a sarcoplasmic protein. It appears progressively over the ageing period.

Sequence of the 90 kDa fragment. An 80% similarity of the sequence was matched with glycogen phosphorylase, a sarcoplasmic protein involved in glycolysis. The origin of this fragment during the ageing period had up to now been uncertain.

Sequence of the 110 kDa fragment. This sequence yielded an 80% match with human skeletal C-protein. The role of C-protein in the myofibril is unknown but it seems to serve as a clamp to hold bundles of myosin filaments together. If so, its degradation during ageing is highly significant because of its proposed structural role. The 110 kDa fragment appears progressively over the tenderisation process.

Other fragments sequenced included the 44 kDa fragment which yielded evidence of its origin as creatine kinase, a 23 kDa fragment as glyceraldehyde-3-phosphate dehydrogenase and a 21.5 kDa fragment as vinculin.

CONCLUSIONS

- The temperature of holding the beef carcass for the first 10 hours post-mortem is critical for tender meat. This applies even when cold-shortening does not occur.
- Meat of high final pH (above 6.2) is more tender than meat of normal final pH (between 5.4 and 5.9), but meat with an intermediate final pH (5.9 to 6.2) is tough.
- The rate at which pH falls early post-mortem influences its ageing response. Fast falling pH muscles are more tender. This probably accounts for some of the variation in tenderness between carcasses.
- Aitch bone hanging of carcasses improves the tenderness of the striploin, topside, silverside and rump.
- Electrical stimulation reduces the risk of cold-shortening but double electrical stimulation does not improve tenderness of beef.
- Beef aged on the bone was not more palatable than beef aged in vacuum packs.
- Calcium ion injection accelerates the tenderisation process but the advantage diminishes with ageing of the beef.
- High pressure has the potential to tenderise meat but needs further development before consideration as a commercial process.
- A relatively small amount of proteolysis occurs during the tenderisation process.

OVERALL CONCLUSIONS

Major improvements in meat tenderness can be made by the careful monitoring of pH and temperature of carcasses at the early post-mortem period. Aitch bone hanging is a simple method to significantly improve tenderness. Tenderisation occurs through the action of proteases on specific substrates of the muscle fibre. Variability of meat tenderness is partly due to the rate of rigor development. Methods to control or predict this rate could be of immense benefit to beef processors.

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