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CHANGES IN THE ULTRASTRUCTURE OF BEEF MUSCLE AS INFLUENCED BY ACIDIC CONDITIONS BELOW THE ULTIMATE pH

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Abstract

Ultrastructural changes in meat incubated with different concentrations of acetic acid were investigated by transmission electron microscopy. Discs of bovine M. longissimus dorsi (48 h postmortem) were incubated at 4°C for 48 h in various acetic acid solutions, giving discs with pH values ranging from 5.1 to 3.9. Below pH 4.5, the volume of the discs increased markedly due to water absorption.

Discs incubated in 0.01M acetic acid had a pH of 5.1, lower swelling ratios and shorter sarcomeres than control samples at pH 5.5. Structural studies of the samples at pH 5.1 revealed that A-band length decreased whereas I-band length increased. There was fragmentation of thin filaments in the Z-line region and disorganisation of Z-line structure.

In discs treated with 0.05M acetic acid (meat pH 4.5), complete loss of M-lines was observed. Noticeable swelling also occurred across the muscle fibre axis, mainly in the A-band region, and myosin filaments were partially extracted. Z-lines were swollen, disorganised and slightly fragmented.

Below pH 4.5, swelling became much more pronounced with further extraction of myosin filaments. Most of the actin filaments were extracted and myofibrils fused together giving an amorphous, coagulated appearance. Z-lines were partially extracted.

In general, the greatest amount of swelling occurred across the muscle fibre axis although significant increases in sarcomere length were observed at pH 4.5 and below.

Cooking of control and 0.01M acid treated meat discs at 80°C increased the space between myofibrils whereas minimal shrinkage was noticed in samples below pH 4.5.

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Introduction

A knowledge of muscle structure is a prerequisite to the successful study of meat as a food (Cassens et al., 1984). In addition to light microscopy, techniques such as transmission electron microscopy (TEM) and scanning electron microscopy (SEM) have proved useful in accurately localizing the structural components of meat products. Such information is useful in developing new products and helping to optimise the composition of existing products (Schmidt, 1984). Ultra-structural investigations of meat emulsions (Ackerman et al., 1971) and of massaged meat (Theno et al., 1978 a, b, c) have been of considerable help in understanding what happens during the processing of sausages and certain sectioned and formed products such as hams.

The pH of meat is extremely important since it has a major influence on the colour, water-holding capacity (WHC), texture and tenderness of meat (Dutson, 1983). Miles and Lawrie (1970) found a positive linear relationship between muscle pH and the tenderness of cooked rabbit muscle over the pH range 5.5 to 7.1. Studies on mutton (Bouton et al., 1971, 1972 a, b) and beef (Bouton et al., 1973 a, b) not only confirmed this relationship between pH and cooked meat tenderness, but also identified a linear increase in WHC over the same pH range. More recently, Gault (1985) identified a progressive increase in the WHC of beef muscle over the pH range 4.6 to 3.0 which coincided with a marked increase in the tenderness of the meat after cooking. Kotula and Heath (1986) also found that the tenderness of chicken was substantially increased by lowering the pH of the meat to 4.5.

In spite of its importance to meat tenderness, little attention had been given to the relationship between WHC and muscle ultrastructure until Offer and Trinick (1983) hypothesised that gains or losses of water in meat are due to the swelling or shrinkage of myofibrils brought about by the expansion or shrinkage of the constituent myofilament lattices. They observed maximum swelling when a substantial part of the A-band had been removed. Studies of blocks of porcine

M. longissimus dorsi (Voyle et al., 1984) revealed that meat incubated in a salt and pyrophosphate solution at pH 8.0 showed complete extraction of the A-bands. However, in meat incubated in the same solution at pH 5.5, extraction of the A-bands was incomplete and there was no extraction of the A-bands at pH 5.5 in salt solution alone.

The ultrastructure of meat at pH values below the isoelectric point (IEP) has not been examined although further acidification of meat is a common feature of the marinading process (Gault, 1984; Kotula and Heath, 1986) and of the manufacture of some varieties of dry sausages (Terrel, 1977). The objective of this study was to investigate and document ultrastructural changes in acidified discs of bovine M. longissimus dorsi covering the pH range 5.1 to 3.9. It was over this pH range that Gault (1985) observed the major changes in meat tenderness in his studies on the relationship between WHC and cooked meat tenderness in acid treated beef muscles.

Materials and Methods

Preliminary treatment of muscles

One M. longissimus dorsi from each of two 18 month old Simmental heifers was obtained 48 h postmortem from a local abattoir. Methods described by Gault (1985) were used for the preparation and acidification of meat discs. Muscles were cut transversely on a gravity feed slicer into steaks approximately 1.0 cm thick, from which discs of meat 3.0 cm in diameter were prepared using a cork borer. Muscles and blocks were tempered at -25°C until the surfaces were rigid in order to facilitate slicing and preparation of discs.

Adjustment of muscle pH

Acetic acid solutions of 0.01, 0.05, 0.10 and 0.25M were used to adjust the pH of the meat discs from pH 5.1 to 3.9. Each meat disc was accurately weighed and placed in a 200 ml capacity screw-cap polystyrene jar to which 50 ml of chilled (4°C) acetic acid solution was added. Penetration of acid into the meat was aided by continuous swirling at 120 rpm in a Gallenkamp cooled orbital incubator for 48 h at 4°C. Untreated meat discs were used as controls. Six discs of meat were selected from each muscle for each treatment, making a total of twelve discs per treatment in all.

Measurement of pH and swelling ratios

After acidification, meat discs were surface dried with paper towelling and reweighed. The pH of all discs was measured directly using a digital pH meter fitted with a spear-type combination glass electrode. Six discs from each treatment were individually cooked in sealed polythene bags in a water bath at 80°C for 20 min and immediately chilled in an ice-water bath. They were then surface-dried as before and reweighed. The weights of each meat disc before and after cooking were divided by the corresponding raw (non-marinaded) meat weight to express changes in weight as swelling ratios which served as measures of WHC in both the raw and cooked discs.

An analysis of variance was carried out on the results obtained for muscle pH and swelling ratios. Mean values and the overall standard errors (s.e.) of these means are presented.

Transmission electron microscopy procedures

Samples measuring approximately 5 x 2 x 2 mm were dissected from the interior of four raw and four cooked meat discs selected from each treatment. These samples were then fixed in acidified 2.5% glutaraldehyde solution for 2 h. They were then transferred to acidified 5% sucrose solution and stored at 4°C for further processing. The pHs of these glutaraldehyde and sucrose solutions were initially adjusted with 1M acetic acid to give an identical pH range to that found in each of the acidified meat discs.

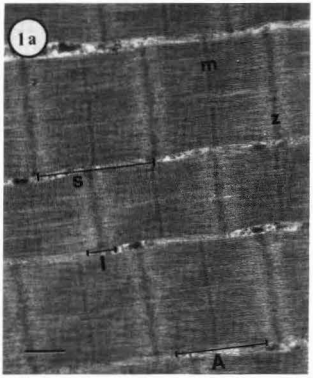
Samples were post-fixed in 1% osmium tetroxide for 2 h. Dehydration through graded alcohols was followed by impregnation in propylene oxide and Durcupan medium resin. The tissues were then embedded in fresh Durcupan resin in coffin moulds and cured in an oven at 60°C for 18 h.

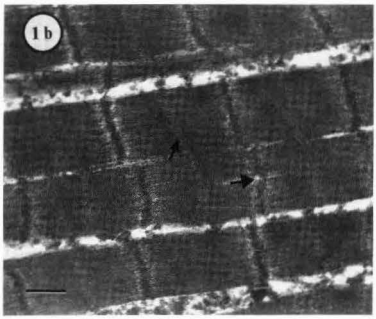
Thick sections (1 µm) were cut from each block and stained with toluidine blue for light microscopy examination. Selected areas from each block were then raised on a LKB pyramitome and ultrathin sections (50 nm) were cut with glass knives on a LKB ultramicrotome. These sections were stained with uranyl acetate for 15 min followed by lead citrate for 5 min.

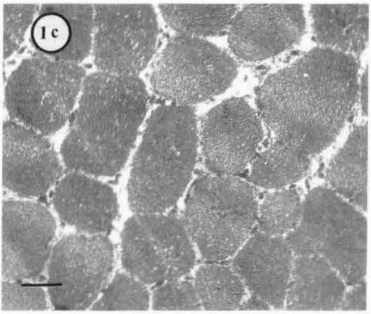
The sections were examined using a JEOL 1200 EX electron microscope and representative areas were photographed. Approximately 50 sarcomere lengths were measured from the photographic negatives of longitudinal muscle sections from each treatment using the macro-system of a LEITZ TAS PLUS computerised image analyser. Unfortunately, the measuring system of the image analyser was not programmed to give a standard deviation and so it was only possible to calculate the mean sarcomere length for each treatment. This was possible throughout the entire pH range studied because of the relative stability of the Z-lines to the various acid treatments. However, it was not possible to measure all myofibril diameters accurately from either transverse or longitudinal muscle sections since adjacent myofibrils fused together to give an overall amorphous appearance in those samples treated with 0.10 and 0.25M acetic acid. Consequently, no attempt was made to measure changes in myofibril diameters.

Results and Discussion

Table 1 summarises the effects of different acetic acid treatments on the pH, swelling ratios and sarcomere lengths of the meat discs. The mean pH of the raw meat discs decreased progressively with increasing acid concentration. Mean swelling ratios (WHC) increased considerably with decreasing pH except in those meat discs closest to the IEP







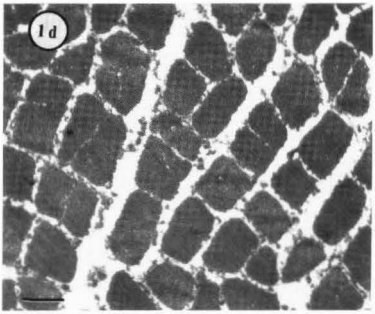


Table 1. Influence of acetic acid concentration (M) on the pH, raw meat swelling ratios (RMS), cooked meat swelling ratios (CMS) and sarcomere lengths (SL) of discs of beef M. longissimus dorsi.

	Control	0.01	0.05	0.10	0.25	s.e.
pH	5.54	5.10	4.48	4.30	3.92	0.014
RMS	0.94	0.90	1.39	1.77	2.04	0.026
CMS	0.60	0.48	1.07	1.45	1.74	0.021
SL (µm):						
raw	1.69	1.56	1.98	2.41	2.56	-
cooked	1.51	1.38	1.76	2.32	2.43	-

of the myofibrillar proteins at pH 5.1, where decreased swelling was observed. These results support the findings of Hamm (1960) and Gault (1985).

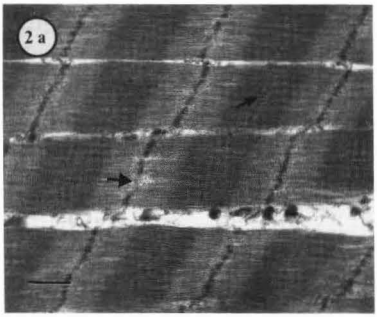
Fig. 1a. Control, raw muscle (pH 5.54). A: A-band; I: I-band; Z: Z-line; m: M-line; S: sarcomere length. (Bar = 0.5µm).

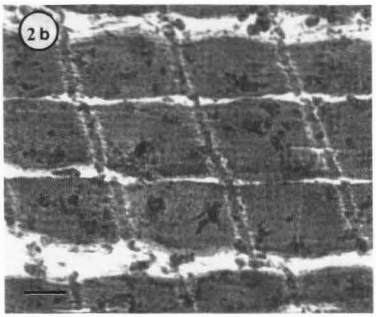
Fig. 1b. Control, cooked muscle. Note the loss of M-lines (small arrow), fragmentation of Z-lines (big arrow) and shrinkage of myofibrils compared to raw muscle (Fig. 1a). (Bar = 0.5µm).

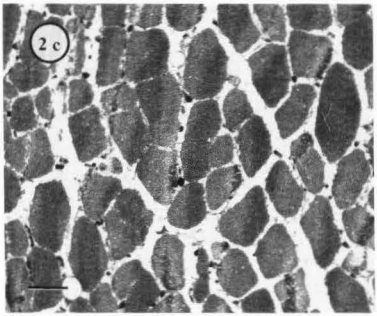
Fig. 1c. Control, raw muscle (pH 5.54). (Bar = 1.0µm).

Fig. 1d. Control, cooked muscle. (Bar = 1.0µm).

Table 1 also indicates that the mean sarcomere lengths varied in a similar manner with changing pH, the shortest sarcomeres being found at pH 5.1. It is interesting that Harrel et al. (1978) also found that beef muscle at pH 4.9 had shorter sarcomere lengths than control samples of normal pH_u. At all pHs cooking resulted in a decrease in both WHC and sarcomere length.







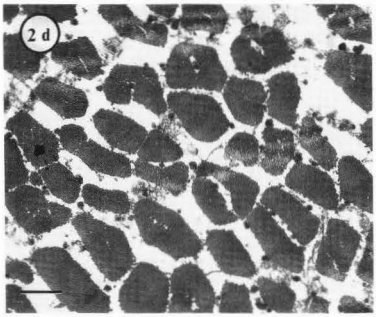


Fig. 2a. Acidified muscle at pH 5.10 - raw. Note the shrinkage of myofibrils, partial loss of M-line structure (small arrow) and disorganised Z-lines (big arrow). (Bar = 0.5μm).

Fig. 2b. Acidified muscle at pH 5.10 - cooked. Note the similarity to Fig. 1b except that myofibrils have shrunk more. (Bar = 0.5μm).

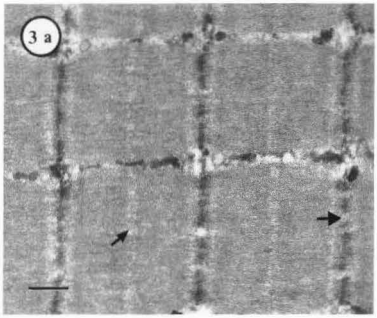
Fig. 2c. Acidified muscle at pH 5.10 - raw. (Bar = 1.0μm).

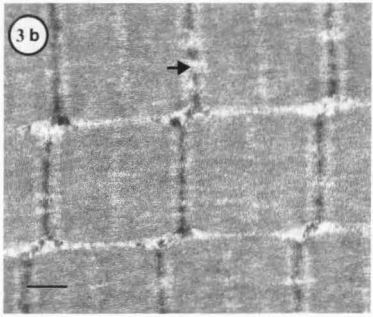
Fig. 2d. Acidified muscle at pH 5.10 - cooked. (Bar = 1.0μm).

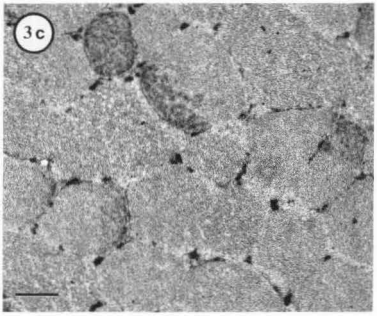
Qualitative details of the accompanying changes in the ultrastructure of these acidified muscle samples are illustrated in Figs. 1-5.

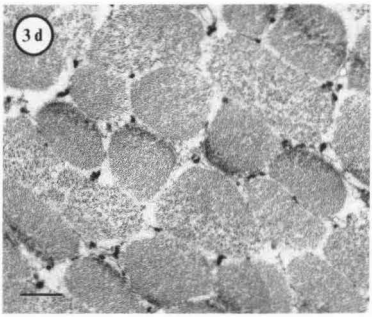
The ultrastructure of the longitudinal and transverse sections of the control samples of raw meat at pH 5.5 are shown in Figs. 1a and 1c, respectively. In Fig. 1a, the very short I-band length compared to that of the A-band is indicative of the short sarcomere length of these muscle samples. Cooking nevertheless resulted in a slight decrease in sarcomere lengths (Fig. 1b), whereas myofibril diameters were greatly reduced (Fig. 1d). Similar changes have also been observed by Paul (1965) and Giles (1969) using light microscopy, and by Schmidt and Parrish (1971) using electron microscopy.

From Fig. 1b it is also clear that cooking of these normal pH_u samples resulted in fragmentation of Z-lines and a complete loss of M-line structure, although pseudo H-zones or bare zones remained visible. A-band filaments were coagulated and there was a partial loss of structure of the I-band filaments. Such observations confirm the results of other studies on cooked beef muscle. Giles (1969), for example, found a complete loss of actin filaments in different beef muscles cooked at 60°C for 20 min and suggested that actin filaments were more heat labile than myosin filaments. Schmidt and Parrish (1971) reported that A-bands became amorphous, detail was lost from Z-lines and the structure of the I-bands was disrupted when beef M. longissimus dorsi was cooked at 90°C.









Muscle samples at pH 5.10 show shrinkage whereas muscle samples at pH 4.48 and below show increased swelling compared to the control muscle samples at pH 5.54.

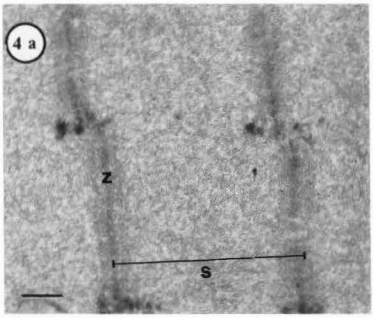
Fig. 3a. Acidified muscle at pH 4.48 - raw. Note the swelling of myofibrils and complete loss of M-line structure. H-zones are still visible (small arrow) and Z-lines are swollen and fragmented (big arrow). (Bar = 0.5μm).

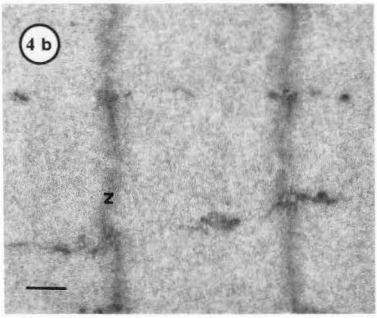
Fig. 3b. Acidified muscle at pH 4.48 - cooked. Z-lines show increased fragmentation (arrow) compared to raw muscle at same pH (Fig. 3a). (Bar = 0.5μm).

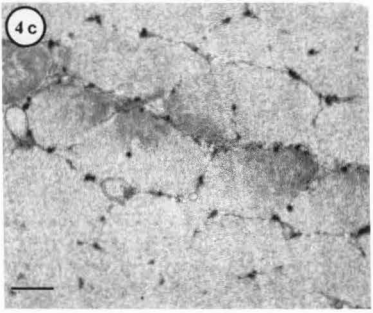
Fig. 3c. Acidified muscle at pH 4.48 - raw. (Bar = 1.0μm).

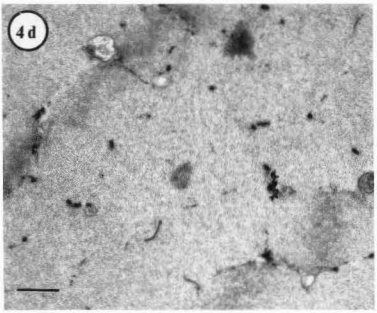
Fig. 3d. Acidified muscle at pH 4.48 - cooked. (Bar = 1.0μm).

In the raw samples at pH 5.1 (Fig. 2a), Z-lines were partially disorganised and fragmentation of the thin filaments was observed in the region of the Z-lines in comparison to the corresponding samples at pH 5.5. There was also some loss of M-line structure. It is also interesting that the length of the A-bands appeared to be smaller, while that of the I-bands appeared to be longer than in the corresponding samples at pH 5.5. These latter changes could have been due to a partial loss of myosin at the A/I band junctions. However, this explanation is most unlikely since solubility studies of beef muscle samples around the IEP showed no dissolution of the major myofibrillar proteins (Gault and Tolland, unpublished work). The most likely explanation, therefore, is that longitudinal as well as transverse shrinkage of the actomyosin filaments occurred at the IEP as they attained their most compact structure, thereby pulling the thin filaments away from the Z-lines. The tension generated by this shrinkage in adjacent sarcomeres may have been sufficient to bring about the observed fragmentation of the thin filaments in the region of the Z-lines as well as the partial disorganisation of the Z-lines discussed above. When these muscle samples were cooked (Fig. 2b), there was a complete loss of M-line









structure and the A-band and I-band filaments became amorphous in appearance.

Over the pH range 4.5 to 3.9, raw meat samples showed a progressive increase in both sarcomere length and myofibril diameter (Figs. 3a, c; 4a, c; 5a, c) compared to both the control samples at pH 5.5 and those at pH 5.1. Such was the extent of swelling across adjacent myofibrils at pH 4.3 and 3.9 that they fused together giving an overall amorphous appearance in both longitudinal (Figs. 4a, 5a) and transverse (Figs. 4c, 5c) sections. Cooking of these samples resulted in a slight decrease in both sarcomere length and myofibril diameter (Figs. 3b, d; 4b, d; 5b, d). However, the shrinkage brought about by cooking in this pH range was not as extensive as that brought about by cooking those samples at pH 5.5 and pH 5.1 as seen by the absence of gaps between the myofibrils of cooked samples at pH 4.5 and below (Figs. 3d, 4d, 5d).

A more detailed examination of the ultrastructure of raw meat samples at pH 4.5 (Fig. 3a) shows quite clearly that M-line structure has been completely lost. It would also appear that much of the myofibrillar

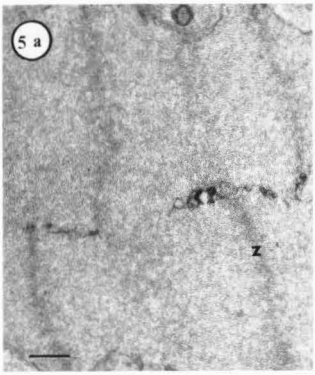
Fig. 4a. Acidified muscle at pH 4.30 - raw. Adjacent myofibrils have fused together and extraction of A-band and I-band filaments has occurred giving an amorphous appearance. Z-lines partially extracted (Z). (Bar = 0.5μm).

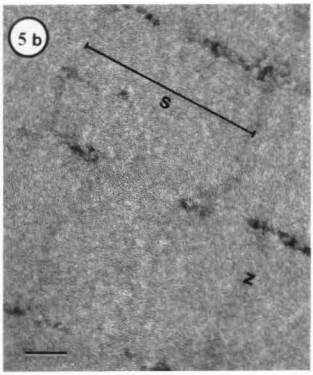
Fig. 4b. Acidified muscle at pH 4.30 - cooked. Similar to raw muscle at same pH (Fig. 4a). (Bar = 0.5μm).

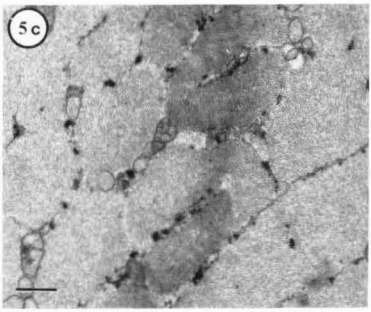
Fig. 4c. Acidified muscle at pH 4.30 - raw. (Bar = 1.0μm).

Fig. 4d. Acidified muscle at pH 4.30 - cooked. (Bar = 1.0μm).

swelling occurred in the A-band region where there was also some evidence of partial extraction of myosin filaments as seen by the partial loss of filamentous structure in the A-band region (Fig. 3a). In contrast, there was comparatively less swelling observed in the I-band region. Z-lines, however, were much more swollen and fragmented than in the higher pH samples. It is likely that the hydrogen ion concentration at pH 4.5 caused a sufficient increase in the net positive charge of the M-line proteins to render them completely







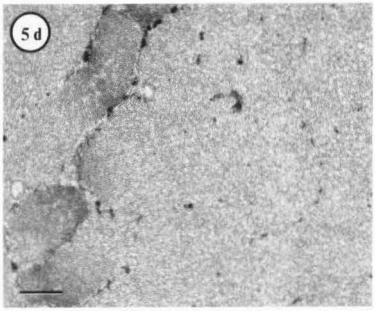


Fig. 5a. Acidified muscle at pH 3.92 - raw. Note further loss of myofibrillar structure and Z-line material compared with raw muscle at pH 4.30 (Fig. 4a). (Bar = 0.5μm).

Fig. 5b. Acidified muscle at pH 3.92 - cooked. Note further loss of Z-line structure (Z) compared to raw muscle at same pH (Fig. 5a). (Bar = 0.5μm).

Fig. 5c. Acidified muscle at pH 3.92 - raw. (Bar = 1.0μm).

Fig. 5d. Acidified muscle at pH 3.92 - cooked. (Bar = 1.0μm).

soluble, thereby removing the structural restraint to myofibrillar swelling from the centre of the A-band. It is also likely that depolymerisation of myosin filaments had begun at the same pH while the actin filaments and Z-line material remained relatively insoluble, showing only restricted swelling. On cooking

Note the fusion of adjacent myofibrils in samples at pH 4.30 and pH 3.92.

(Fig. 3b), further fragmentation of Z-lines was observed with little effect on the other myofibrillar ultrastructural details.

In those raw meat samples at pH 4.3, further myofibrillar swelling was observed and adjacent myofibrils were seen to fuse together (Fig. 4a). H-zones were completely lost for the first time, indicating complete depolymerisation of actomyosin and dispersion of myosin filaments. There was also further extraction of A-band and I-band filaments giving an amorphous appearance throughout each sarcomere. Z-lines were partially extracted and the remaining Z-line structures were seen to fuse together to form a longitudinal band across adjacent myofibrils. Cooked samples had a similar appearance except in the proximity of the Z-lines which were comparatively shrunken (Fig. 4b).

There was evidence of extensive swelling and further loss of structure in the raw meat samples at pH 3.9 (Fig. 5a) with only faint traces of Z-line material remaining. These alterations were more severe in cooked samples (Fig. 5b) where the Z-lines became barely visible.

The ultrastructural changes brought about by the acidification of these samples over the pH range 4.5 to 3.9 clearly indicate the severe disruptive nature of low pH on the myofibrillar components of meat. Nevertheless, most of these changes, which involved myofibrillar swelling and dissolution, are similar to those observed in individual myofibrils by Offer and Trinick (1983) and by Voyle et al. (1984) in meat blocks treated with various combinations of salt and pyrophosphate solutions in the pH range 5.5 to 8.0.

Offer and Trinick (1983) hypothesized that the removal of one or more transverse structural constraints, such as the actin/myosin cross-bridges, the M-lines or the Z-lines, by salt and pyrophosphates, would play an important role in myofibrillar swelling by allowing the filament lattices to enlarge. The loss of M-line structure was observed by Voyle et al. (1984) in pork muscles incubated with salt and pyrophosphate solutions for 24 h. However, in samples incubated for 5 h, M-lines were still seen. Similar observations on the variable appearance of the M-lines in meat treated with polyphosphates has been attributed by Lewis et al. (1986) to calcium availability, since these authors have shown that the addition of calcium resulted in a complete loss of M-line structure. Porzio et al. (1979), who studied the extraction and isolation characteristics of different myofibrillar proteins, have also indicated that the M-line proteins are the weakest link in the structural strength of myofibrils in raw muscle. In our studies, we have observed a complete loss of M-line structure at pH 4.5, indicating that the M-lines are also the most vulnerable structural components to the acid treatment of raw muscle. This loss of M-line structure could explain why swelling was much more extensive in the A-band region than in the I-band region at pH 4.5, particularly since there was only a partial loss and slight fragmentation of Z-line structure at the same pH.

It is also worth emphasising that in the studies of Offer and Trinick (1983), certain myofibrils showed a complete loss of Z-lines only when a critical concentration of salt or salt and pyrophosphate had been reached. However, in other myofibrils subjected to similar treatments (Offer and Trinick, 1983) there was no apparent loss of Z-line material, and in meat treated with salt alone (Lewis et al., 1986), Z-lines could still be seen. In our experiments, we found that Z-lines were the most resistant of all myofibrillar components to acetic acid treatment, and the loss of Z-line structure with increasing acidity was a gradual process. This would have contributed to the greater swelling observed in the I-band region at pH 4.3 and general loss of all structural integrity within the myofibrils, particularly at pH 3.9, where only faint traces of Z-line structure remained.

In conclusion, the extensive swelling observed within the myofibrils of the more acidified samples of beef muscle would appear to be related to the solubility characteristics of the various structural proteins retained within the myofibril. Clearly, some structural components were more resistant to depolymerisation and solubilisation than others. It is, therefore, very likely that the extent of swelling observed was related primarily to the osmotic pressures generated by the presence of a highly charged and concentrated dispersion of myofibrillar proteins. The limited myofibrillar swelling at the higher pHs was a function of the relative integrity of the transverse constraints, the strongest of which were the Z-lines, with the weakest being the M-lines. At pH 5.1, shrinkage of the myofibrils occurred as the constituent myofilament lattices attained their most compact structure at the IEP.

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Discussion with Reviewers

A.-M. Hermansson: How did tempering to -25°C affect the microstructure?

Authors: Previous studies have indicated that freezing of meat causes the formation of ice crystals between muscle fibres and that this brings about fragmentation of myofibres (Voyle, 1979; Martino and Zaritzky, 1986). These studies have also indicated that freezing results in protein denaturation and a loss of water-holding capacity. However, we would like to emphasize that in our studies the muscles were tempered at -25°C in a chill cabinet for 1 h, rather than blast frozen, to facilitate the slicing procedure. Consequently, it was only the peripheral areas of these muscles which were frozen, and the cores of meat which were subsequently used as controls did not show any damage to muscle components when examined either by electron microscopy, as in the present study, or by light microscopy (Rao et al., 1989). We would therefore conclude that the tempering of muscles at -25°C for short periods of time did not have any significant effect on the microstructure of our meat samples.

G.R. Schmidt: Could the addition of from 0 to 0.25M acetic acid have altered the ionic strength and/or the osmolality to produce the swelling observed? Could the varying level of acetate have been standardized by a different experimental design?

Authors: Yes. The addition of these increasing amounts of acetic acid would certainly have increased both the ionic strength and osmolality of the meat discs. We nevertheless feel that the increased swelling was primarily due to the influence of H^+ ions increasing the net positive charge of the myofibrillar proteins below the isoelectric point. However, it is also likely that an osmotic swelling effect may predominate as the pH approaches 4.0, where the dissociation of acetic acid (pKa = 4.7) becomes more suppressed.

The varying level of acetate could have been standardized by using different buffer systems made up with acetic acid and sodium acetate. However, this would alter the ionic balance with respect to sodium and hydrogen ion concentration, particularly at the higher pH values close to the isoelectric point. What effect, if any, this would have on the pH-hydration curves of beef discs is perhaps an area of study worthy of further investigation.

G.R. Schmidt: Were the untreated meat discs which were used as controls also swirled in the orbital incubator?

Authors: The untreated meat discs were placed in the orbital incubator as dry controls, ie. without the addition of distilled water.

D.F. Lewis: How does your process relate to domestic/commercial cooking procedures and how does the structure vary from the inside to the outside of the pieces?
Authors: The cooking procedures were identical to those used by Gault (1985) in his studies on the tenderness of marinaded meat. The procedures used ensured that the internal temperature of the meat discs was maintained at 80°C for 15 min, a satisfactory time/temperature combination to ensure the thermal denaturation of native myofibrillar proteins and collagen.

All our samples for microscopy were dissected from the interior of the meat discs where leaching of myofibrillar proteins into the equilibrating solution would be minimal at the lower pH values. We would, however, expect considerable protein extraction below pH 4.5 from the outer regions of the meat discs. Consequently, we would expect that the structural appearance of the peripheral regions of these meat discs, both before and after cooking, would be extensively disrupted.

D.F. Lewis: What is the effect of fixing control meat in acidified glutaraldehyde, or for that matter, of fixing acidified meat in pH 7.0 glutaraldehyde?
Authors: The rationale behind our approach to the fixation process was to minimise changes in tissue pH brought about by the fixative. Hayat (1970) has also recommended this approach since inadvertent changes in tissue pH brought about by any fixative would undoubtedly alter the structure and behaviour of tissue proteins. Since we were generally looking at changes in muscle structure below the iso-electric point, we felt that it was essential to keep the pH of the glutaraldehyde solutions as close to that of the meat discs as possible.

With regard to the efficiency of our fixation process, it is generally recognised that condensation reactions between aldehyde and amino groups are favoured under mildly acidic conditions. Under the pH conditions used in our work, the imino links formed between bi-functional glutaraldehyde molecules and the amino groups of different protein molecules would be expected to remain stable.

D.F. Lewis: What effect does pH treatment of meat have on the staining characteristics of myofibrillar proteins with uranyl acetate and lead citrate?
Authors: As we appeared to get good contrast in all our sections, we were quite happy with the staining procedure we used.

The staining technique we used was based on post-fixation of samples with osmium tetroxide followed by several dehydration stages before the samples were embedded. Ultra thin sections of embedded tissue were then stained with uranyl acetate followed by lead citrate to enhance the contrast of the osmium post-fixed material. Therefore, we would not expect the original pH of the meat samples to have any effect on the efficiency of the uranyl acetate and lead citrate counterstains.

P.J. Knight: The small change from pH 4.5 to 4.3 appears to cause a dramatic change from filamentous to granular in the appearance of longitudinal sections of raw meat (Figs. 3a and 4a). Is this a consistent effect or is there variation within or between samples?
Authors: Yes. This granular appearance of myofibrils in meat samples at pH 4.3 was consistent in all of the samples we obtained although there were small variations in the appearance of the granular structure between samples.

P.J. Knight: Is the 50% increase in sarcomere length of raw samples after acid treatment reflected in an increase in the thickness of the meat disc?
Authors: We observed a gradual increase in the thickness of raw meat discs treated with 0.05M and higher concentrations of acetic acid. We believe that this increase was certainly due to the lengthening of sarcomeres at pH values below the IEP. However, we did not measure the actual physical dimensions of either the control or the acid treated meat discs.

P.J. Knight: Do the changes in dimensions of the myofibrils on cooking account for the changes in weight of the meat pieces?
Authors: Yes. The extent of longitudinal and transverse shrinkage observed in myofibrils due to cooking was closely associated with the extent of swelling ratio reduction (weight loss) measured for the various meat discs at each particular pH.

We have also carried out detailed light microscopy studies on six different types of beef muscle treated with similar concentrations of acetic acid. These studies showed that both raw and cooked meat swelling ratios were positively correlated ($P<0.001$) with both muscle fibre diameter and sarcomere length, although these correlations were much stronger with fibre diameter than with sarcomere length (Rao et al., 1989).

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