EFFECT OF CALCIUM IONS ON TEXTURE OF BEEF DURING CONDITIONING

A. ALARCON-ROJO AND E. DRANSFIELD

Institute of Food Research, Langford, Bristol BS18 7DY, U.K.

INTRODUCTION

Tenderness is considered one of the main attributes of meat quality and it is enhanced during conditioning. However the mechanism of this process remains unclear. The increase in tenderness is associated with the action of the endogenous proteolytic enzymes, the neutral proteinases or calpains and the acidic lysosomal enzymes. Two types of calpains are known, calpain I which is activated by micromolar calcium concentrations and calpain II which is activated by millimolar concentrations of calcium.

Both enzyme systems have been claimed to reproduce the changes in protein profiles observed during storage of meat (Penny, 1980). It has been suggested that cathepsins could function more effectively than calpains in weakening myofibrils at post rigor conditions (Etherington, 1984), mainly because their degradation is higher at the low pH found in post rigor meat (Dutson, 1983). However, calpains also hydrolyse muscle proteins (Goll *et al.*, 1983) and it has been found that calpain I retains 24-28% of its activity at pH 5.5-5.8 at post mortem conditions (Koohmaraie, *et al.*, 1986). A study on different bovine muscles, suggested that cathepsins have little participation in the tenderising process because Troponin in M. *psoas major* disappeared more slowly than in M. *semitendinosus,* but *psoas major* had higher cathepsin levels (Olson, *et al.*, 1977). Recent work of Koohmaraie *et al.* (1988) has contributed to this controversial matter. They were able to accelerate conditioning of beef by activating calpains with calcium and found that when beef slices were incubated with calcium chloride, most of the post mortem changes took place within 24 hours of incubation; however, a previous claim was that this effect was non-enzymatic (Hattori and Takahashi, 1979). The aim of the present work was to evaluate the effect of calcium levels and calcium salts on the post rigor tenderisation of beef.

MATERIALS AND METHODS

Semitendinosus muscles from 18 month old bovines were excised at 24 h postmortem and cut into 30 mm thick slices across the fibre axis. Each slice was cut into 30x20x4mm strips along the fibre axis, and 100 g of strips were soaked in 250 ml of tris/maleic buffer with a final salt concentration of 100 mM and pH 5.5. To inhibit spoilage, 2ml of 0.1M sodium azide were added per litre of soaking solution. The solutions also contained one of the following agents: 5 to 60 mM calcium chloride, 30 mM calcium acetate, 30 mM calcium nitrate, 30 mM calcium lactate, or 0.1 mM calpain inhibitor (N-acetyl-leu-leu-norleucinal) which inhibits calpain I at .044 mM and Calpain II at .018 mM (Boehringer). In addition, strips were soaked in buffer for 24 hours and then calcium chloride was added (30 mM), or in 0.1mM inhibitor with 30 mM calcium chloride. In the combined solution calcium was added 2 hours after soaking with inhibitor, to allow diffusion of the inhibitor into the meat.

The strips were stored at 10°C for 1, 2, 3, 4, 6 and 8 days. At each conditioning time, samples were taken for cooking and mechanical measurements, the corresponding volume of soaking solution was also removed to keep the meat:solution ratio 1:2.5.

Meat samples, in sealed plastic bags, were cooked in water at 80°C for 20 min, then cooled in running tap water and stored at 4°C overnight.

Toughness was assessed with an Instron TM-SM fitted with the Volodkevich jaws using a crosshead speed of 25 mm/min and a chart speed of 100 mm/min. Cooked meat strips were stacked to give 10 mm square cross section blocks and sheared at right angles to the muscle fibre direction. The force/time curve was recorded and the first yield force, expressed as Kg force calculated by measuring the height of the first peak on the deformation curve.

To remove variation in shear force between animals, the values were expressed as percentage of the control at 1 day after commencing of soaking.

RESULTS

Figure 1 shows the shear force (mean and standard deviation of two animals) of the control and the effect of the specific calpain inhibitor on tenderisation of meat during storage at 10°C. A marked decrease in shear force during the first two days of storage was observed in the control with small further change during the remainder of the storage time. The meat treated with the inhibitor was always tougher than the control. After 24 hours soaking, the inhibitor-treated meat showed shear force values 20% higher than the control and did not change during storage

for up to 6 days.

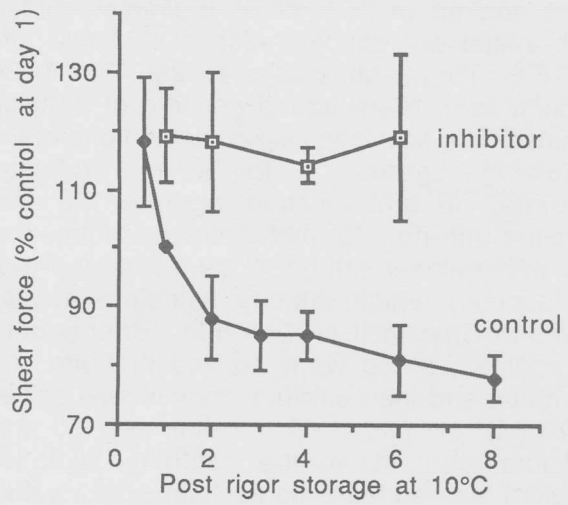

Fig. 1. Effect of inhibitor on tenderisation

The changes in shear force(mean values and standard deviation of two animals) with calcium concentration are presented in Figure 2. The shear force values are the average of the shear force at day 1, 2, and 6 relative to the control at day 1 of storage. When soaked 1 day postmortem, a progressive decline in shear force was found when the calcium chloride concentration of the soaking solution increased proportionally from 5 to 30 mM with very little further change at higher concentrations up to 60 mM. The most marked change was found in the 40 mM calcium chloride-treated meat which had a shear force 50% lower than the control, whereas a slightly higher value was observed in the meat soaked in 50 mM calcium chloride solution.

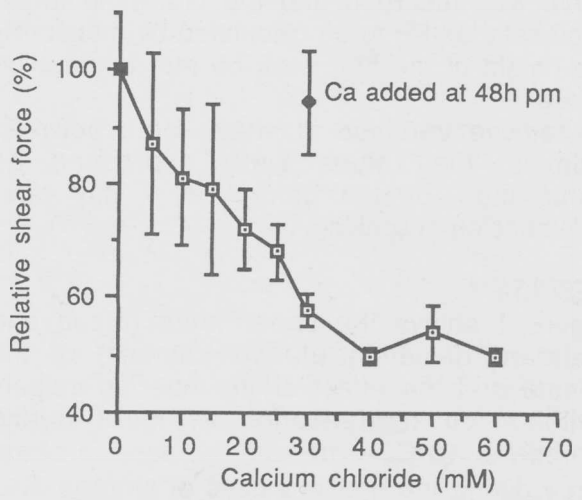

Fig. 2. Effect of Ca concentration on tenderisation

In general, it was found that the maximum tenderising effect of calcium took place at about 30 mM concentration. On the other hand, when 30 mM calcium chloride was added at day 2 of storage, only 6% tenderisation was observed (Fig. 2).

Figure 3 shows the effect of calcium salts and calcium plus inhibitor on tenderisation of meat. The concentration of calcium salts in the soaking solutions was 30 mM and all the salts were able to accelerate the process of tenderisation.

The calcium-treated meats had lower shear force values than the control during the whole conditioning time. Calcium chloride, calcium nitrate and calcium acetate were equally effective in tenderising meat; all of them showed shear force values 40% less than the control at 24 hours of storage with a little further change up to 8 days at 10°C.

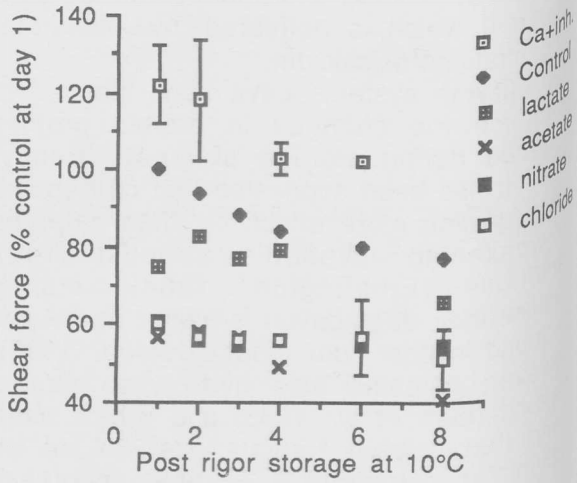

Fig. 3. Effect of calcium salts and inhibitor on tenderisation

Calcium lactate caused little effect on shear force, although there was a 25% decrease in toughness at day 1 of storage, the value at 8 days was similar to that of the control. With inhibitor and calcium chloride solution, the tenderisation was markedly reduced and the treated meat showed shear force values 20% higher than the control during the whole conditioning time.

DISCUSSION

Soaking small strips of post rigor meat was a reliable method to diffuse exogenous salts into the meat structure and it caused less than 10% swelling. This technique was able to reproduce the normal ageing of beef in which an 80% reduction of the shear force would be expected in 4 days of storage at 10°C (Dransfield *et al.*, 1981).

Enzyme inhibitors have been successful in studies of proteolysis *in vivo* (Stracher *et al.*,

1978) and in the involvement of enzymes in myofibrillar disruption (Hattori and Takahashi, 1979). The peptide inhibitor used in the present work was N-Acetyl-leu-leu-norleucinal, which is a substrate-like inhibitor of calpains, and the concentration used was 0.1 mM for optimal inhibitory effect on calpain activity. In addition, this inhibitor did not inhibit cathepsins B and L activity of meat.

The failure of meat to tenderise when the inhibitor was added, clearly indicates that the endogenous degradative system which alters the texture has been blocked. Considering the specificity of the inhibitor to calpains, the importance of such enzymes to the ageing process was demonstrated.

The strong relationship observed between shear force and calcium concentration appears to reveal an important fact on conditioning. Calcium was able to tenderise meat at concentrations as low as 5 mM and as the concentration increased up to 30 mM, the rate of tenderisation was markedly accelerated. Although the magnitude of the tenderisation in normal aged meat was smaller than that caused by calcium, and the intracellular free calcium level of normal skeletal muscle is only 10^{-5} M (Jöbsis and O'Connor, 1966), the present work suggests that in both cases, the mechanism of tenderisation could be similar.

It is well recognized that purified skeletal Calpain I needs micromolar levels of calcium and that Calpain II requires from 1 to 5 mM calcium to be fully activated (Penny, *et al.*, 1985); however, it should be taken into account that these requirements refer always to those of the highly purified enzyme, and that impure enzyme preparations may need higher calcium levels (Mellgren, 1980). An important remark is that most of the studies on calpains are carried out *in vitro* and under optimal proteolytic conditions for such enzymes, such as neutral pH, isolated myofibrils, and using purified proteinase; this is evident in the early reports on the removal of Z-disks by calpains (Dayton *et al.*, 1975).

The data of our experiments suggest that the calcium added could have activated mainly Calpain II due the high levels of calcium present in the meat. Additionally, the probability of Calpain I being also activated is rather low, since it has been found to be more labile to storage than Calpain II (Ducastaing *et al.*, 1985), and all our experiments were carried out after 24 hours post mortem.

The explanation for the relatively high calcium concentration needed to produce the maximum tenderisation of meat, could be that only the free calcium ions are able to activate calpains, and it is possible that in the conditions of this study, some calcium could be bound to proteins or to inorganic groups present in the meat. The results of this experiment indicate that Calpain II was fully activated and that it was able to produce all the tenderisation observed. Moreover, the effect of calcium was restricted to the early conditioning period, *i.e.* calcium tenderised meat only when added within 24 hours postmortem, probably because calpain II was rapidly inactivated by the calcium after full activation. This is supported by the observations that both calpains are autolysed in the presence of calcium with a loss of enzyme activity (Suzuki *et al.*, 1987).

Although 15% reduction in shear force was observed when calcium was added to the inhibitor solution, the treated meat was always more tough than the control over the conditioning period. Therefore, the possibility of calcium having a direct action on the meat texture is very remote, and it is suggested that most of the tenderising effect of calcium is by stimulating calpain II. Contrarily, Hattori and Takahashi (1979) believed that the effect of high concentrations of calcium on myofibrils is non-enzymatic. The basis of their conclusion was that calcium showed higher myofibrillar fragmentation than the purified calpain, and iodoacetate, which would inhibit cysteine proteinases, did not stop fragmentation. However, they also observed that homogenization alone was able to produce fragmentation of myofibrils. Therefore, the disagreement of their work with ours is based on the experimental methods used.

The observation that different calcium salts produced similar effects on texture, suggests that calcium is the ion involved in the tenderisation process, and that the anion is irrelevant to such changes. The exception was the lactate which showed slightly lower tenderising effect than the other salts, but the reason of this is not yet very clear.

In conclusion, this study presented evidence of the potential implication of calcium in tenderising meat in the early post rigor period.

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