ULTRASTRUCTURE AND MYOFIBRILLAR STRENGTH OF *PRE-* OR *POST-RIGOR* $CACL_2$ INJECTED MEAT.

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S-IVB.12

SUMMARY

Changes in the ultrastructure and myofibrillar resistance of *pre-* or *post-rigor* calcium chloride-injected *Longissimus* muscle have been analysed at different ageing times. Injections of 10 % w/w of 0.1 M or 0.3 M $CaCl_2$ were performed in 2.5 cm-thick steaks either in the *pre-rigor* (pH = 6.9, 6.3 or 5.9) or *post-rigor* (pH = 5.4) stages. Untreated steaks, 0.15 M or 0.6 M NaCl-injected steaks and water-injected steaks were used as controls. *Pre-rigor* injections of calcium lead to an intense shortening of the myofibrils; myofilaments were immediately very disorganised. However, these disturbances were only observed two hours after the injection of the lower amount (0.1 M $CaCl_2$) of calcium. No further change was evidenced during the ageing process. Mechanical resistance of raw myofibrils reached its lowest value at one day or two days *post mortem* when injected with 0.3 M or 0.1 M $CaCl_2$ respectively. Similar results were observed after injections performed at later *pre-rigor* stages (at pH = 6.3 or even at pH = 5.9). In *post-rigor* treated samples, no ultrastructural change was noticed immediately after injection. However sarcomere fragmentation and decrease in resistance of raw myofibres at day 2 occurred to a greater extent than in the control. NaCl- or water-injected samples were similar to control regarding ultrastructure and mechanical properties of raw myofibres, except those injected with a high amount of sodium (0.6 M) at pH = 6.9 which significantly decreased the resistance of raw myofibres during the first two days after the treatment. These results suggest that the modification of ultrastructure and resistance of raw myofibres induced by calcium injection in the *pre-rigor* stage is probably not due to an enhancement of enzymatic process, whereas *post-rigor* injection may accelerate the enzymatic mediated ageing process.

Introduction

Tenderness of beef meat is generally judged to be the most important quality required by the consumer. As tenderness is submitted to a great variability, largely due to differences in the weakening of myofibrillar proteins for animals of a similar age (Koohmaraie, 1994), technological treatments are needed to provide tenderization of meat and to limit the variability. Tenderization of meat can be completed by one day *post mortem* by infusion or injection of a $CaCl_2$ solution in the *pre-rigor* period (30 min *post mortem*) (Koohmaraie *et al.*, 1990 ; Morgan *et al.*, 1991 ; Wheeler *et al.*, 1991). Recently Wheeler *et al.* (1993) demonstrated that 5% (w/w) of a 0.2 M $CaCl_2$ solution injected in *post-rigor* beef muscles was the optimal amount to improve tenderness in some muscles and also minimize off-flavor potential that is generally induced by *pre-rigor* or to a lesser extent by *post-rigor* treatments with higher amount of $CaCl_2$. However, the mechanisms involved in calcium-induced tenderization are still not clear. Two phenomena are often opposed : the Ca^{++}-induced weakening of Z-disks (Takahashi *et al.*, 1987; Takahashi, 1992) and the removal of Z-disks by calpains, calcium-dependant proteases (Koohmaraie *et al.*, 1988; Koohmaraie, 1994). In *post mortem* muscle cell, the amount of calcium is raised to 0.1 mM. This concentration of Ca^{++} is sufficient to activate μ-calpains but not m-calpains (Etherington, 1984 ; Ouali, 1990). Therefore calcium injected into muscle may activate the enzymatic proteolysis induced by both μ-calpains and m-calpains. Koohmaraie *et al.* (1988) noted that calpains could be preserved from autolysis in the presence of Ca^{++}-chelators, whereas in Ca-treated samples autolysis occurred. Furthermore if Ca^{++} is substituted by Zn^{++}, a calpain inhibitor, all weakening or solubilization of myofibrillar proteins is inhibited (Taylor and Etherington, 1991). But the calcium-induced weakening of Z-disks, if it is not excluded by some authors (Koohmaraie *et al.*, 1989 ; Wheeler *et al.*, 1990 ; Aalhus *et al.*, 1993 ; Koohmaraie, 1994), is demonstrated by others. Takahashi (1992), in experiments on

isolated myofibrils incubated with Ca^{++} and calpastatin, concluded that weakening is non enzymatically induced. A salting-in of specific proteins could occur in the presence of divalent metal ions (Ca^{++}, Mg^{++}) (Taylor and Etherington, 1991).

The objective of this research was to study the changes in the myofibrillar ultrastructure that occur in $CaCl_2$-injected meat and to compare the ultrastructural *post mortem* modifications induced by *pre-rigor* or *post-rigor* treatments. These ultrastructural changes have been linked to the mechanical behaviour of raw treated meat.

Materiel and methods

Animals. Four Friesian young bulls (24 to 26 months old) and five Friesian cull cows (5 to 10 years old), of 250 to 340 kg carcass weight, were slaughtered according to standard procedures.

Muscles. Muscles *Longissimus thoracis* and *lumborum* (LD) from the two half-carcasses were excised immediately after slaughter, cut in 2.5 cm-thick steaks, treated and vacuum-packed or vacuum-packed until treatments, stored in a 15°C water bath until 24 h *post mortem* and then at 4°C until measurements. Treatments were randomly assigned to each of the 2.5 cm-thick steaks.

Treatments. Steaks were injected with 10 % of a 0.1 M or 0.3 M $CaCl_2$ solution either in the *pre-rigor* period (at pH = 6.9 essentially, 6.3 or 5.9 in some experiments) or in the *post-rigor* stage (at pH = 5.4; 24 h *post mortem*). Controls consisted of untreated steaks, 0.15 M or 0.6 M NaCl-injected muscles and deionized-water-injected muscles. These solutions were injected with 10% (w/w) either in the *pre-rigor* (at pH = 6.9) or *post-rigor* (at pH = 5.4) stages. The samples were injected with an eight needle (0.6 x 25 mm) multi-pipette at various and regular locations to ensure an homogeneous distribution of solutions.

Ultrastructural changes in muscle cells. Ultrastructural changes in control and treated muscles have been analysed by observation of longitudinal sections of muscle in transmission electron microscopy, immediately after the treatments (at d 0 or at d 1) and at different ageing stages (at d 1, d 2, d 7 and d 14). Muscle samples (10 x 5 x 5 mm) were fixed by immersion in 2.5 % glutaraldehyde in 0.1 M sodium cacodylate buffer, pH = 7.2, for 4 hours at 4°C. Small blocks (3 to 5 mm^3) were post-fixed in 1 % osmium tetroxide in sodium cacodylate buffer 0.1 M, pH = 7.2, for 1 hour at 4°C. The blocks were dehydrated through an ethanol gradient and embedded in epoxy resin (TAAB, Eurobio France). The ultra-thin sections (70 nm) were stained with uranyl acetate and lead citrate, then observed with a Philips EM400 electron microscope under an acceleration voltage of 80 kV.

Sarcomere lengths. Sarcomere lengths were measured at d 7 by diffraction of a laser beam according to the method of Cross *et al.* (1980), on samples fixed in glutaraldehyde solutions. Lengths of 30 diffraction patterns were measured in each sample.

Myofibrillar resistance on raw meat. Kinetics of myofibrillar resistance decline were performed on raw meat from *rigor* onset (at d 0 or d 1) to 14 days *post mortem* (at d 2, d 7 and d 14). Myofibrillar resistance was analysed by compressing raw meat samples up to a maximum 0.2 strain (Lepetit *et al.*, 1986). Compression was performed perpendicularly to the myofibre axis in a sinusoidal compression cycle (0.1 second period) using the Food Texture Analysis System (Salé *et al.*, 1984), or by a linear compression using a universal testing machine (INSTRON). Results presented here have been corrected according to Lepetit and Buffiere (1993) in order to get values of the same order of magnitude with the two methods.

Results and discussion

Pre-rigor injections of calcium

Ca 0.3 M - pH = 6.9. Injections with 0.3 M $CaCl_2$ performed at day 0 (pH = 6.9) exhibited immediate changes in the ultrastructure of the myofibres (figure 1). Z-line structures were missing very soon after the treatment in some areas. Myofilaments were very disorganised, the thick filaments seemed to be partly solubilised. The myofibrils were enlarged, probably because of the high contraction, about 42 %, sarcomere length 1.1-1.2 μm (Table 1), of the myofibres that occurred just after the injection. A great decrease, of about 60 %, in the myofibrillar resistance was observed as soon as *rigor* was set up (figure 3). No further modification was evidenced along the ageing period (d 2, d 7 and d 14). However in some places, and sometimes in the same myofibres, the strong shortening did not occur and in this case only, the sarcomere fragmentation was similar to the untreated control (figure 1). During the ageing process, myofibrillar resistance of *pre rigor* 0.3 M $CaCl_2$-treated samples was quite stable from *rigor* onset to d 14, with a lowest strength obtained one day after the

injection (d 1). Wheeler *et al.* (1991) and Morgan *et al.* (1991) also demonstrated the complete tenderization of meat by one day *post mortem* after such a calcium treatment. However, the final strength (at d 14) of treated samples was 20 % lower than that of the untreated controls. In parallel to these ultrastructural and mechanical studies, sensory assessments have also been performed on aged meat. Whereas disturbances observed on raw meat were very strong, tenderness analysed by a trained panel was very low as compared to controls or *post-rigor* calcium treated samples (results not shown). This was probably due to the strong contraction which is only detectable after cooking.

Ca 0.3 M - pH = 6.3, pH = 5.9. Injections of 0.3 M $CaCl_2$ performed at pH = 6.3 or pH = 5.9 gave the same apparent results in muscle ultrastructure as those obtained when the injection was performed at pH = 6.9. As previously, a strong shortening occurred in the myofibrils just after the injections, even after the injection at pH = 5.9 where the intracellular stores of calcium are supposed to be released in the fibres (data not shown). Again myofibrillar resistances significantly decreased to a higher extent in treated samples as compared to the untreated ones, especially at day 2 (figure 3).

Ca 0.1 M - pH = 6.9. A lower amount of calcium (0.1 M $CaCl_2$) injected early in the *pre-rigor* period (at pH = 6.9) gave the same ultrastructural observations as for the corresponding 0.3 M $CaCl_2$-injection. However, the disturbing effects on the myofibrillar structure did not appear immediately after the treatment but occurred only 1 to 2 hours after the injection period, probably because calcium diffuses towards the myofibrils during this time (figure 1). The shortening occurred to the same extent as previously (Table 1). Kinetics of decline in myofibrillar resistance followed a similar pattern to that obtained for the corresponding 0.3 M $CaCl_2$ injection, but with a slightly less marked effect (figure 3).

Post-rigor injections of calcium

Ca 0.1 M, Ca 0.3 M - pH = 5.4. Whatever the concentration of injected calcium (0.3 M or 0.1 M $CaCl_2$), the treatments in the *post-rigor* stage (at pH = 5.4 ; 24 h *post mortem*) gave the same apparent ultrastructural pattern. Just after the injections, no contraction was observed and no relevant changes in the myofibrillar structure were noticed. However in some fibres a strong weakening of the Z-lines already occurred at day 1, these disturbances could account for an accelerated proteolysis. But the extent of the sarcomere fragmentation was not amplified during the ageing period (figure 2). Sarcomere lengths (1.9 μm) were not modified by the treatment (Table 1). Kinetics of myofibrillar resistance decrease during ageing gave no significant differences between controls and treated samples (figure 3).

Controls

Pre-rigor or *post-rigor* injected samples (0.6 M or 0.15 M NaCl and water) were used as controls for the *pre-rigor* or *post-rigor* calcium treatments (0.3 M or 0.1 M $CaCl_2$). No relevant modifications in the myofibrillar ultrastructure were observed following these control injections as compared with the corresponding calcium injections (figure 2). Sarcomere lengths were not modified (Table 1). No positive effects on the decrease in myofibrillar strength were noticed, except a significant one for the *pre-rigor* 0.6 M NaCl-injection on the first days (at d 2) after the treatment (figure 3). The slight effect observed after the injection of a high amount of NaCl in the *pre-rigor* stage may suggest that the effect of calcium was not a specific effect. The increase in ionic strength could have helped for all the modifications observed, but could not account for all the disturbances observed.

Conclusion

Pre-rigor injection of 0.3 M $CaCl_2$ (at pH = 6.9, pH = 6.3 or even at pH = 5.9) lead to great and immediate disturbances in the myofibrillar ultrastructure and mechanical behaviour of raw meat. A lower amount of injected calcium (0.1 M $CaCl_2$) lead to similar but slightly delayed effects. The immediate and not progressive effects observed after the *pre-rigor* treatments may suggest that calcium injected *pre-rigor* could not account for an accelerating proteolysis. Positive effect on raw myofibre resistance may not be found in sensory analysis as contraction effect is detectable only after cooking as in the case of cold-shortening. *Post-rigor* injections did not lead to similar significant results. The effects of ionic strength have to be distinguished from the effects of enzymatic proteolysis as concluded by Wu and Smith (1987). The effects on ageing of ionic strength and enzymatic proteolysis may be synergistic.

Acknowledgements

Thanks are expressed to B. Dominguez, R. Labas, J.F. Chazeix and J.F. Gardette (I.N.R.A.) for technical assistance.

This work was supported by the EU-AIR Programme (Project : CT92-0521).

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