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Myofibrillar structural changes caused by marination with calcium phosphate or calcium chloride and sodium pyrophosphate

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MYOFIBRILLAR STRUCTURAL CHANGES CAUSED BY MARINATION WITH CALCIUM PHOSPHATE OR CALCIUM CHLORIDE AND SODIUM PYROPHOSPHATE

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Summary

Ultrastructural changes were studied in beef eye of round muscle after 120 hours marination in 0.5, 0.75, or 1.0% calcium phosphate (CaPO) or 2, 4, or 6% calcium chloride or 1% sodium pyrophosphate (CaCl+NaPO) solutions. Increasing the concentration of CaPO caused decreasing myofibril width and increasing myofilament degradation. Increasing the concentration of CaCl+NaPO caused increasing loss of I-band material. Marination of beef eye of round muscle in calcium phosphate or calcium chloride + sodium pyrophosphate solutions caused denaturation of myofibrillar proteins likely due to marinating solution acidity.

(Key Words: Marinades, Muscle Structure.)

Introduction

Variability in meat tenderness is a major quality defect of current beef production practices. Marination can add various ions to muscle to alter pH and ionic strength, ultimately affecting the degree of muscle fiber disruption. Injecting beef and lamb muscles with calcium chloride ($CaCl_2$) improves tenderness through the activation of calcium-dependent proteases as well as a salting-in of the calcium ions, causing degradation of Z-disk proteins (desmin and nebulin), titin, C-protein, troponin-I, troponin-T, and tropomyosin. Phosphate compounds increase water-holding capacity by increasing negative charge electrostatic repulsion, and improve tenderness by dissociating actomyosin. Each of these can lead to improved tenderness. Our objective was to investigate changes in the ultrastructure of beef caused by marination in either calcium phosphate or calcium chloride-sodium pyrophosphate solutions.

Experimental Procedures

We used the eye of round muscle from one side of a commercially fed and slaughtered beef animal. At 40 hours postmortem, the muscle was sliced into 1-inch steaks perpendicular to muscle fiber orientation. These steaks were further sliced into small muscle samples with final dimensions of .2 inch × .4 inch × 1.0 inch. The longitudinal axis of the muscle fibers coincided with the long axis of the small tissue samples. Three calcium phosphate ($Ca(H_2PO_4)_2$) solutions (0.50, 0.75, and 1.0% calcium phosphate monobasic), 3 calcium chloride ($CaCl_2$) + sodium pyrophosphate ($Na_2H_2P_2O_7$) solutions (2, 4, and 6% calcium chloride plus 1% disodium pyrophosphate), and a no liquid, unmarinated control were utilized to test the effects of marination on the small muscle samples. Each tissue sample was placed in a 15 ml conical polystyrene centrifuge tube to which 10 ml of a single marination solutions was added deionized water. The pH of each marination solution was

also recorded. After 120 hours of marination, final sample pH was determined by homogenizing five samples per treatment in their respective solutions and measuring pH of the homogenate. After marination, three samples were randomly selected from each treatment for transmission electron microscopy. Microscopy samples (.04 inch × .04 inch × .11 inch) were cut from the muscle blocks using a razor blade, fixed in 3% gluteraldehyde - 0.1*M* sodium cacodylate for three hours under slight agitation on an orbital shaker, rinsed three times in 0.1*M* sodium cacodylate - 0.1*M* sucrose, and post-fixed in 1% osmium tetroxide - 0.1*M* sodium cacodylate for 2 hours (1 hour in ice, 1 hour at room temperature). Samples were then rinsed three times with distilled water, dehydrated using a graded acetone series (50, 70, 80, 90, 100, 100, 100%), infiltrated with resin, placed in embedding molds, and heated (140°F) for 48 hours to polymerize the resin. Thin sections were cut using glass knives on an ultramicrotome and mounted on 250-mesh copper grids before electron micrographs were taken. pH data were analyzed as a one-way treatment structure in a repeated measures experimental design. Marination solution was the between-subject treatment factor and the initial and after marination measurements were the within-subject repeated measures. Differences among treatments and between repeated measures were tested and separated when significant ($P<0.05$).

Results and Discussion

Increasing concentrations of calcium phosphate (CaPO) or calcium chloride-sodium pyrophosphate (CaCl+NaPO) decreased solution pH (Table 1). Calcium phosphate is an acidulant used in the baking industry, therefore the low pH was expected. However, the low pH of the CaCl+NaPO solutions was unexpected. Apparently, both calcium chloride and sodium pyrophosphate dissociated, then the calcium complexed with the pyrophosphate, liberating hydrogen ions and decreasing solution pH.

Measurements of pH (Table 1) indicated the control increased ($P<0.05$) in pH unmarinated while the 0.75 and 1.0% CaPO and all CaCl+NaPO treatments decreased ($P<0.05$) in pH. No significant difference between initial and final pH was detected for the 0.5% CaPO treatment. Muscle tissue pH of each treatment was similar initially; however, a stepwise decrease in tissue pH after marination was noted as CaPO or CaCl+NaPO concentration increased, reflecting the acidic properties of the calcium phosphate and calcium chloride solutions and loss of muscle tissue buffering capacity.

Figures 1 through 7 illustrate the myofibrillar structure of *semitendenosis* muscle samples subjected to each of the 7 treatments. In Figure 1 (control) the Z-lines were intact and clearly defined. The A-bands and I-bands had no noticeable degradation and the H-zone was detectable. Overall, sarcomere components had clear definition and were aligned in a systematic manner. In Figure 2 (0.50% CaPO), only slight degradation of Z-lines was detected as noted by a few gaps or breaks. The Z-lines were less intact and less in register than the control and the H-zone was still detectable. A noticeable loss of Z-line stability seen in Figure 3 (0.75% CaPO), indicates significant structural degradation. The Z-lines were in less register at the lower concentration and wavy appearance had increased. The H-zone was not well defined, indicating less structural integrity amongst the myosin filaments. In Figure 4 (1.0% CaPO) increased Z-line degradation occurred compared to the 0.75% CaPO treatment. The H-zone was barely detectable and the Z-lines were in disarray, indicating significant structural alteration. In addition, both the 0.75% and 1.0% CaPO

treatments caused narrowing of the myofibrils compared to the negative control, likely due to the loss of water caused by acid denaturation of the myofilaments and the sarcoplasmic reticulum. The 2% CaCl + 1% NaPO treatment (Figure 5) caused the Z-lines and H-zones to lose some structural order. However, the H-zone and I-bands were clearly identifiable. In Figure 6, muscle samples marinated in 4% CaCl + 1% NaPO had partially degraded Z-lines due to their loss of order and appearance of multiple gaps or breaks. In addition, less I-band material was seen, indicating increased degradation of actin filaments. Muscle marinated in 6% CaCl +1% NaPO (Figure 7) also showed many gaps and loss of Z-line order. The I-band was less visible, suggesting more structural alteration of I-band material from this treatment.

Our original hypothesis was that low concentrations of calcium phosphate would dissociate in water, yielding free calcium and phosphate. Free calcium was anticipated to enhance calpain activity, thereby increasing structural degradation of the myofibrillar component, while the phosphates would bind water. However, even low concentrations of calcium phosphate dissociated poorly in water. Calpain activity was likely not enhanced because of the poor dissociation of the marination compounds as well as the low pH of the marination solutions. A synergism was expected to exist between calcium chloride and sodium pyrophosphate, but they apparently dissociated and calcium complexed with pyrophosphate, thereby decreasing pH. We suggest that degradation evident in the micrographs resulted from acid denaturation of myofibrils and possibly from increased activity of cathepsin enzymes.

Beef eye of round marinated in a calcium phosphate or calcium chloride - sodium pyrophosphate solution showed myofibrillar protein structural changes when compared to non-marinated controls. The changes were likely the result of acid denaturation of proteins. In applied practice, we do not recommend the use of calcium phosphate or calcium chloride-sodium pyrophosphate solutions for marination of beef.

Table 1. pH of Marination Solutions and Eye of Round Tissue Samples Initially and 120 Hours after Marination in Calcium Phosphate or Calcium Chloride + Sodium Pyrophosphate Solutions (S.E.=0.067)

Marination Treatment	pH Solution	Initial pH	Final pH
Untreated control	----	5.42^{b}	$5.67^{a,t}$
0.50% Calcium phosphate	4.53	5.38^{a}	$5.43^{a,u}$
0.75% Calcium phosphate	4.37	5.39^{a}	$5.16^{b,v}$
1.00% Calcium phosphate	4.30	5.47^{a}	$4.94^{b,w}$
2% Calcium chloride + 1% Sodium pyrophosphate	2.90	5.44^{a}	$3.55^{b,x}$
4% Calcium chloride + 1% Sodium pyrophosphate	2.71	5.25^{a}	$3.21^{b,y}$
6% Calcium chloride + 1% Sodium pyrophosphate	2.39	5.43^{a}	$2.83^{b,z}$

^{a,b}Means within a row with different superscripts differ ($P<0.05$).
^{t,u,v,w,x,y,z}Means within a column with different superscripts differ ($P<0.05$).

Effect of Treatment on Muscle Structure

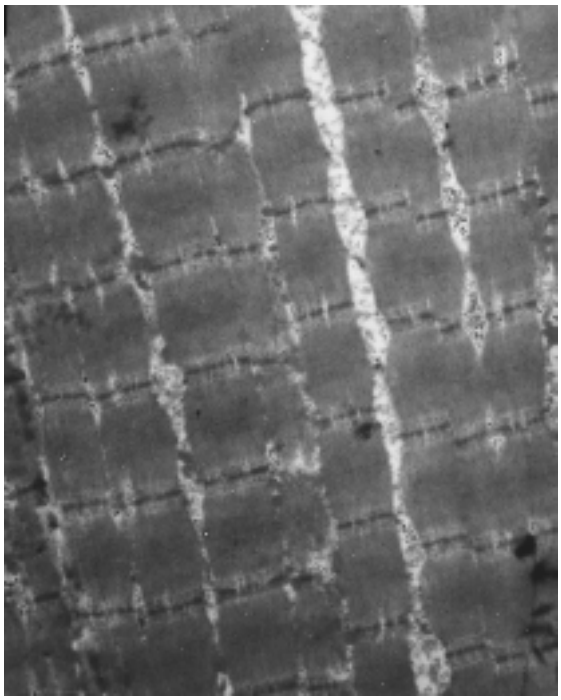

Figure 1. Control

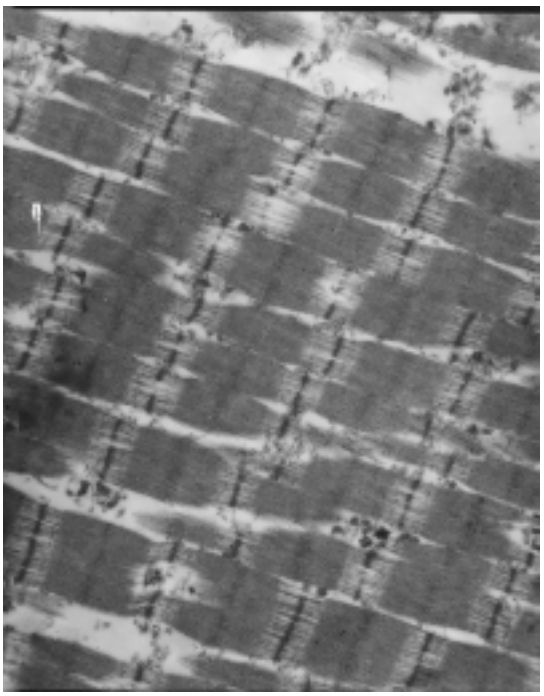

Figure 2. 0.50% Calcium phosphate

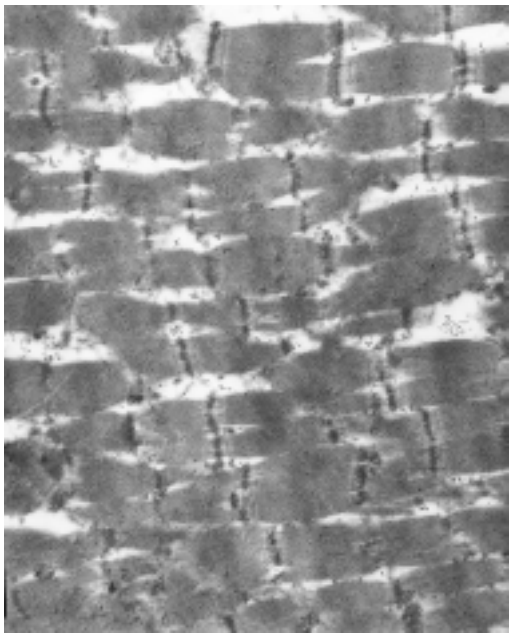

Figure 3. 0.75% Calcium phosphate

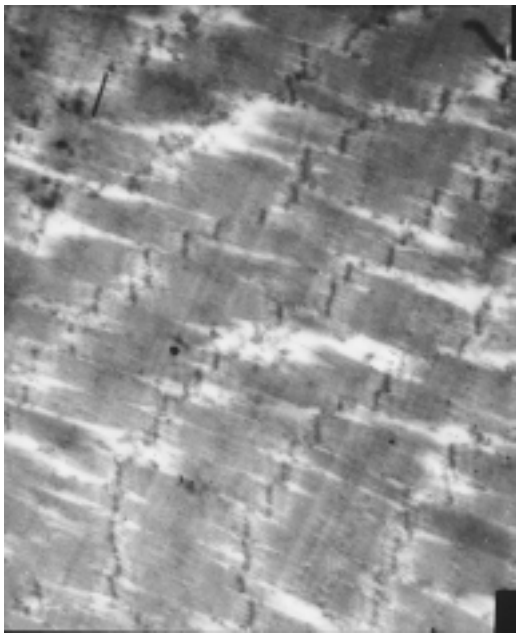

Figure 4. 1.0% Calcium phosphate

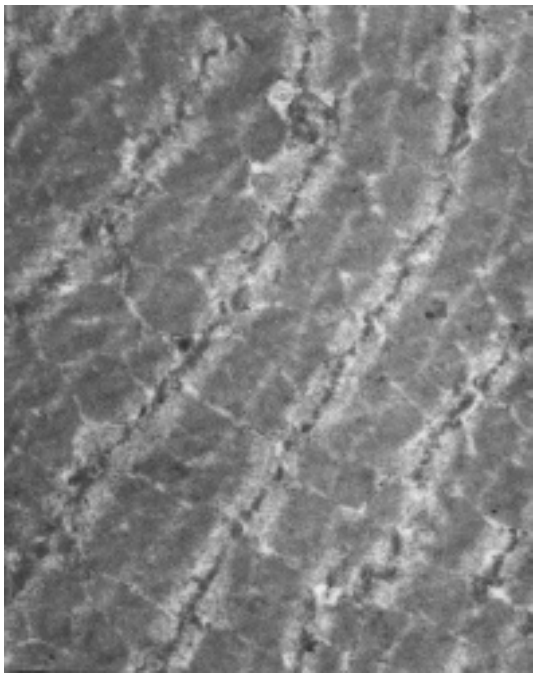

Figure 5. 2% Calcium chloride + 1% Sodium pyrophosphate

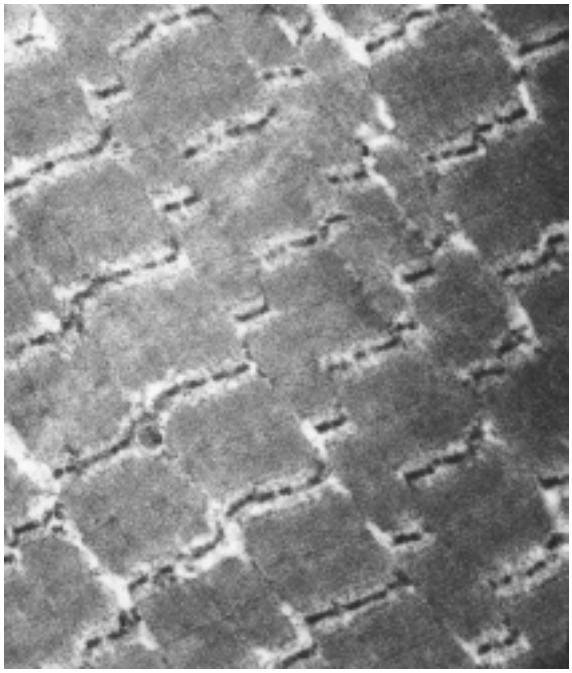

Figure 6. 6.4% Calcium chloride + 1% Sodium pyrophosphate

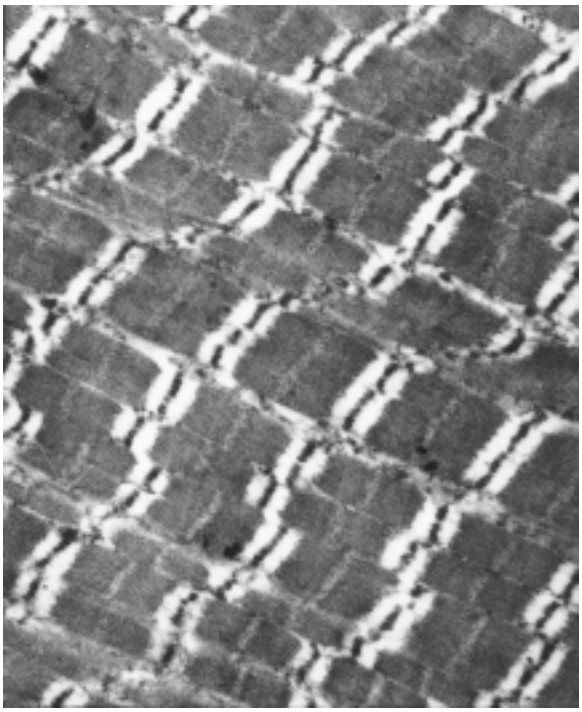

Figure 7. 6% Calcium chloride + 1% Sodium pyrophosphate