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## Effects of injection marination with various calcium sources and molar concentrations on display color life, tenderness, and microbial inhibition of beef loin steaks

### Abstract

Beef strip loins were assigned to one of 11 treatments that included injection marination (10% by weight) with three calcium salts at three molar concentrations, a distilled water control, and a non-marinated control. The effects of calcium salt and concentration were tested for retail display color life, tenderness and sensory traits, and microbial growth. Calcium lactate marinated steaks had longer color life and less microbial growth than those treated with calcium chloride or calcium ascorbate. Increasing molar concentration (.1M to .2M to .3M) caused faster color deterioration, and did not significantly improve microbial inhibition. All calcium treatments improved tenderness; however, calcium chloride treatments induced off-flavors. Considering a whole system approach that accounts for color life, microbial inhibition, shear force, and sensory traits, we recommend injecting beef longissimus with 10% of a .1M solution of calcium lactate, and do not recommend other calcium salts or concentrations.

### Keywords

Cattlemen's Day, 2002; Kansas Agricultural Experiment Station contribution; no. 02-318-S; Report of progress (Kansas State University. Agricultural Experiment Station and Cooperative Extension Service); 890; Beef; Marination; Calcium; Color life; Tenderness; Microbial inhibition; Loin steaks

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**EFFECTS OF INJECTION MARINATION WITH VARIOUS  
CALCIUM SOURCES AND MOLAR CONCENTRATIONS ON  
DISPLAY COLOR LIFE, TENDERNESS, AND MICROBIAL  
INHIBITION OF BEEF LOIN STEAKS**

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**Summary**

Beef strip loins were assigned to one of 11 treatments that included injection marination (10% by weight) with three calcium salts at three molar concentrations, a distilled water control, and a non-marinated control. The effects of calcium salt and concentration were tested for retail display color life, tenderness and sensory traits, and microbial growth. Calcium lactate marinated steaks had longer color life and less microbial growth than those treated with calcium chloride or calcium ascorbate. Increasing molar concentration (.1M to .2M to .3M) caused faster color deterioration, and did not significantly improve microbial inhibition. All calcium treatments improved tenderness; however, calcium chloride treatments induced off-flavors. Considering a whole system approach that accounts for color life, microbial inhibition, shear force, and sensory traits, we recommend injecting beef longissimus with 10% of a .1M solution of calcium lactate, and do not recommend other calcium salts or concentrations.

**Introduction**

Numerous researchers have cited the benefit of injecting beef and lamb with calcium chloride to improve tenderness. Despite the improvement in tenderness, calcium chloride has caused bitter, metallic, and livery off-flavors. In addition, calcium chloride injected muscle is often darker and discolors faster than untreated muscle. No research has been published on the injection of calcium ascorbate to improve tenderness, and only

one report exists on the use of calcium lactate. Our objective was to investigate the effects of calcium ascorbate, calcium chloride, and calcium lactate at three molar concentrations on beef loin steak color life, shear force and sensory traits, and microbial inhibition.

**Experimental Procedures**

At 40 hours postmortem, USDA Select beef strip loin subprimals (n=26) were trimmed of all external fat and accessory muscles, and cut into three equal sections. Loin sections were randomly allocated to one of the following treatments: injection (10% by weight), of calcium ascorbate, lactate or chloride, each at .1, .2 or .3M; 10% distilled water, and a non-marinated control. Each loin section was vacuum packaged, tumbled for 15 minutes, and stored at 32°F until 14 days postmortem.

At 14 days postmortem, two 1-inch steaks for shear force and sensory panel evaluations, and two 0.75-inch steaks, one for simulated retail display and the other for initial microbial assessment, were cut from each loin section. Shear force and sensory panel steaks were vacuum packaged, and frozen until evaluations began. Retail display steaks were placed on white foam trays and overwrapped with oxygen permeable PVC film (23,250 cc/m<sup>2</sup>/24 h). Steaks were displayed for three days at 37°F under 150 foot candles of Philips Ultra-lume™ fluorescent lighting in a retail

display case and visually evaluated by trained color panelists at 0 (before display), 24 and 48 hr using a scale of 1 = very bright cherry red, 2 = bright cherry red, 3 = slightly dark red to tannish red, 3.5 = borderline acceptable, 4 = moderately grayish tan to brown, 5 = tan to brown. Shear force and sensory panel steaks were cooked on a MagiKitch'n electric belt grill set at 242°F, to 158°F internally. Half-inch diameter cores were taken parallel to muscle fiber orientation with a mechanical coring device, and cores were sheared once through the center by a V-notch Warner-Bratzler shear attachment connected to an Instron Universal Testing Machine. Sensory panel steaks were evaluated for myofibrillar and overall tenderness (1 = extremely tough, 8 = extremely tender), juiciness (1 = extremely dry, 8 = extremely juicy), flavor intensity (1 = extremely bland, 8 = extremely intense), connective tissue amount (1 = abundant, 8 = none), and off-flavor intensity (1 = abundant, 8 = none). Two surface cores (1.0 inch diameter, ~1/8 inch thick) were aseptically cut from the microbiological samples, added to 99 ml of 0.1% peptone buffer, and stomached for 2 minutes. Initial (at start of display) and final (after 5 days of display) samples were plated at  $1$ ,  $10^{-1}$ ,  $10^{-2}$ , and  $10^{-3}$  in duplicate on Aerobic Plate Count Petrifilm™ and incubated for 48 hours at 96°F. Microbiological growth was counted and converted to log<sub>10</sub> colony forming units per cm<sup>2</sup>.

The treatment structure was a 3 × 3 factorial (3 calcium salts × 3 molar concentrations) with negative (non-marinated) and positive (distilled water) controls. The design structure was an incomplete block design. Loin was the blocking factor and one-third of a loin was the experimental unit. The statistical model included the fixed effect of treatment, and the random effects of loin and location within loin (anterior, middle, posterior). Treatment means were generated and separated when

significant ( $P < 0.05$ ). In addition, single degree of freedom contrasts were used to test the main effects of calcium salt and molar concentration.

## Results and Discussion

Color deterioration varied with calcium source and molar concentration (Figure 1). The vertical dotted line represents the color limit of consumer acceptance; to the right of the line is unacceptable meat color. Color of calcium ascorbate injected steaks was unacceptable or approached unacceptability at 0 time ( $P < 0.05$ ). Regardless of calcium salt, injection of a .3M solution usually caused a less ( $P < 0.05$ ) desirable color than a .2M or .1M solution. Treatments with the most acceptable color were .1M calcium chloride, .1M calcium lactate, and distilled water, all of which had color scores <2.5 after 48 hours of display. Ascorbic acid can have both antioxidant and prooxidant effects. Apparently all levels of calcium ascorbate that we tested encouraged pigment oxidation. The increasing level of color deterioration (oxidation) due to molar concentration is likely due to an increased level of metal ions (calcium), which donate free electrons to oxidation reactions.

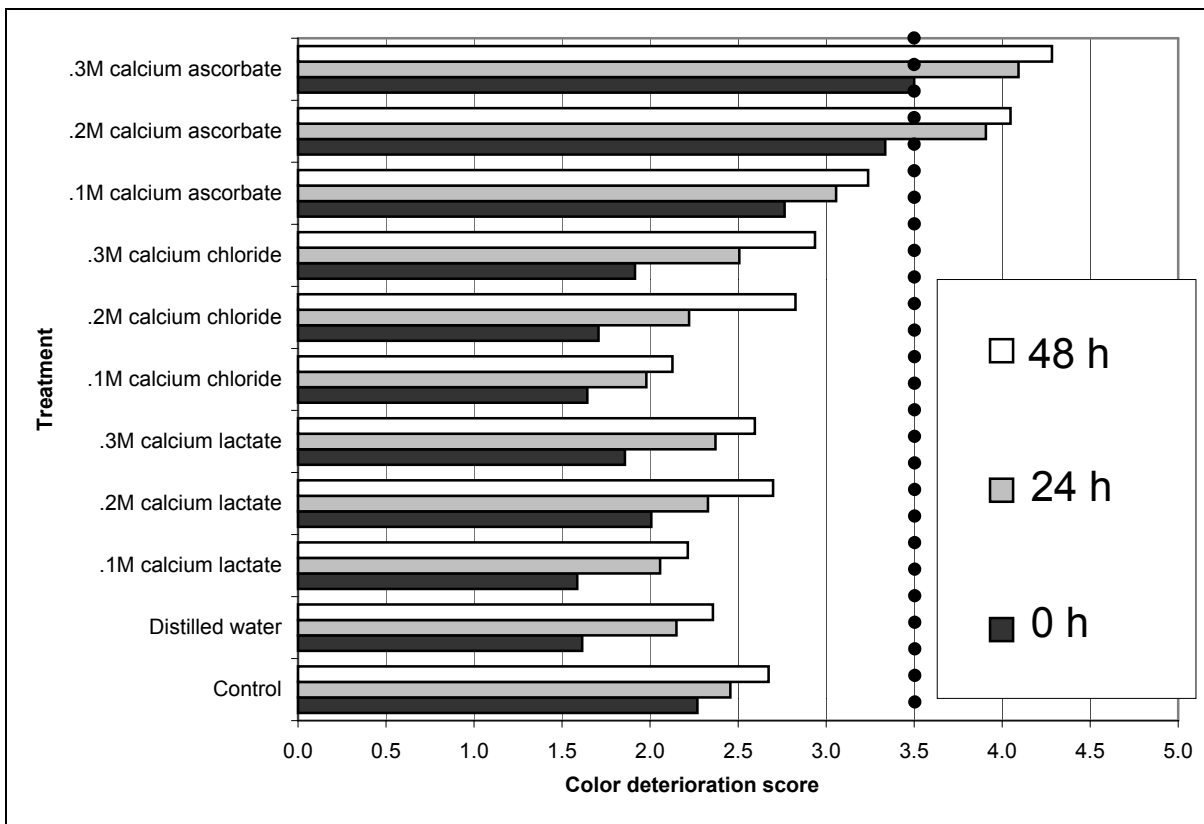
As expected, all calcium treatments reduced shear force values by 24.3 to 41.6%, compared to the non-marinated control (Table 1). In addition, calcium marination improved myofibrillar and overall tenderness compared with both controls, and connective tissue amount scores compared with the non-marinated control. Because longissimus muscle was used throughout this experiment, differences in sensory connective tissue scores are likely not real differences, but perceived differences based on variation in myofibrillar toughness. Juiciness scores were not different across treatments. Beef flavor intensity scores were highest for calcium lactate and lowest for calcium chloride treatments.

Off-flavor intensity scores were poorest for .3M calcium chloride (often characterized as bitter, metallic, sour, soapy, and astringent), followed by .2M calcium chloride and .3M calcium ascorbate.

All calcium sources initiated calcium-induced tenderization. Even the lowest molar concentration met the minimum requirement for calpain enzyme activation. Furthermore, calcium salts other than calcium chloride improved tenderness.

Initial aerobic plate counts were similar among treatments; however, all counts except for .2M calcium lactate increased ( $P<.05$ ) after 5 days of simulated retail

display (Table 2). Beef loin steaks treated with .3M calcium lactate, .2M calcium chloride, or .2M calcium lactate had lower ( $P<0.05$ ) counts than steaks treated with .1M calcium chloride, .2M calcium ascorbate, or .3M calcium chloride. Because there is no consistent pattern, it is difficult to recommend a specific calcium source or concentration based on microbial characteristics. Based on the data presented here, to improve beef longissimus tenderness and palatability traits without having a detrimental effect on display color life, we recommend injection with a 10% solution of 0.1M calcium lactate.



**Figure 1. Visual Color Scores of Calcium Marinated Beef Loin Steaks.**

**Table 1. Warner-Bratzler Shear Force (lbs) and Sensory Panel Scores of Loin Strips Injected with Three Calcium Salts at Three Molar Concentrations**

Treatment	Warner-Bratzler Shear Force	Myofibrillar Tenderness	Juiciness	Beef Flavor Intensity	Connective Tissue Amount	Overall Tenderness	Off-Flavor Intensity
.3M Calcium ascorbate	5.48 <sup>a</sup>	6.51 <sup>d</sup>	5.32 <sup>a</sup>	4.82 <sup>a b c</sup>	7.40 <sup>d</sup>	6.69 <sup>d</sup>	5.81 <sup>b</sup>
.2M Calcium ascorbate	6.15 <sup>a b</sup>	6.01 <sup>b c d</sup>	5.32 <sup>a</sup>	4.90 <sup>b c</sup>	6.98 <sup>b c</sup>	6.20 <sup>b c d</sup>	6.89 <sup>c</sup>
.1M Calcium ascorbate	6.65 <sup>a b</sup>	5.81 <sup>b</sup>	4.83 <sup>a</sup>	4.95 <sup>b c</sup>	6.99 <sup>b c</sup>	6.09 <sup>b</sup>	7.23 <sup>c</sup>
.3M Calcium chloride	5.87 <sup>a b</sup>	6.36 <sup>c d</sup>	5.11 <sup>a</sup>	4.50 <sup>a</sup>	7.32 <sup>c d</sup>	6.62 <sup>c d</sup>	4.87 <sup>a</sup>
.2M Calcium chloride	6.38 <sup>a b</sup>	5.93 <sup>b c</sup>	5.03 <sup>a</sup>	4.69 <sup>a b</sup>	7.07 <sup>b c d</sup>	6.15 <sup>b c</sup>	6.13 <sup>b</sup>
.1M Calcium chloride	6.80 <sup>a b</sup>	5.80 <sup>b</sup>	5.13 <sup>a</sup>	5.05 <sup>b c</sup>	7.05 <sup>b c d</sup>	6.11 <sup>b</sup>	7.01 <sup>c</sup>
.3M Calcium lactate	6.35 <sup>a b</sup>	6.41 <sup>c d</sup>	5.22 <sup>a</sup>	5.02 <sup>b c</sup>	7.19 <sup>c d</sup>	6.59 <sup>b c d</sup>	7.41 <sup>c</sup>
.2M Calcium lactate	6.71 <sup>a b</sup>	6.06 <sup>b c d</sup>	5.29 <sup>a</sup>	5.18 <sup>c</sup>	7.21 <sup>c d</sup>	6.34 <sup>b c d</sup>	7.40 <sup>c</sup>
.1M Calcium lactate	7.10 <sup>b</sup>	5.88 <sup>b c</sup>	5.22 <sup>a</sup>	5.16 <sup>c</sup>	7.06 <sup>b c d</sup>	6.17 <sup>b c</sup>	6.93 <sup>c</sup>
Distilled water	8.55 <sup>c</sup>	5.00 <sup>a</sup>	4.94 <sup>a</sup>	4.92 <sup>b c</sup>	6.77 <sup>b</sup>	5.28 <sup>a</sup>	7.41 <sup>c</sup>
Control	9.38 <sup>c</sup>	4.70 <sup>a</sup>	5.07 <sup>a</sup>	5.02 <sup>b c</sup>	6.31 <sup>a</sup>	5.00 <sup>a</sup>	7.44 <sup>c</sup>

<sup>a,b,c,d,e</sup>Within a column, means with a common superscript letter do not differ (P<0.05).

**Table 2. Aerobic Plate Counts ( $\log_{10}$  CFU/cm<sup>2</sup>) of Marinated Beef Loin Steaks Initially and after 5 days of Simulated Retail Display**

Treatment	Initial	Final
.3M Calcium ascorbate	1.31 <sup>y</sup>	2.91 <sup>a b c d, z</sup>
.2M Calcium ascorbate	1.41 <sup>y</sup>	3.47 <sup>c d, z</sup>
.1M Calcium ascorbate	0.90 <sup>y</sup>	2.96 <sup>a b c d, z</sup>
.3M Calcium chloride	1.32 <sup>y</sup>	3.71 <sup>d, z</sup>
.2M Calcium chloride	0.94 <sup>y</sup>	2.15 <sup>a, z</sup>
.1M Calcium chloride	1.53 <sup>y</sup>	3.28 <sup>b c d, z</sup>
.3M Calcium lactate	1.14 <sup>y</sup>	2.13 <sup>a, z</sup>
.2M Calcium lactate	1.36 <sup>y</sup>	2.18 <sup>a, y</sup>
.1M Calcium lactate	1.46 <sup>y</sup>	2.37 <sup>a b, z</sup>
Distilled water	1.11 <sup>y</sup>	2.61 <sup>a b c, z</sup>
Control	1.60 <sup>y</sup>	2.19 <sup>a, y</sup>

<sup>a,b,c,d</sup>Within a column, means with a common superscript letter do not differ (P<0.05).

<sup>y, z</sup>Within a row, means with a common superscript letter do not differ (P<0.05).