Effect of Postmortem Injection Time and Postinjection Aging Time on the Calcium-Activated Tenderization Process in Beef

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ABSTRACT: The objectives of this study were to determine 1) the effectiveness of calcium chloride when injected later than 2 d postmortem, 2) the effect of extended postinjection aging time, and 3) the tenderness response curve in calcium chloride-treated beef. In Exp. 1, the longissimus thoracis et lumborum was injected on either d 2 or 14 postmortem with 5% by weight of a 200 mM calcium chloride solution. Samples were aged (1°C) either 7 or 35 d after injection. The uninjected control longissimus from the contralateral side was aged for 9, 21, 37, or 49 d. In Exp. 2, the longissimus thoracis et lumborum was injected on d 2 postmortem with 5% by weight of a 200 mM calcium chloride solution then sampled for shear force on d 1, 2, 6, 8, 12, and 14 after injection. Calcium chloride injection, regardless of injection time or postinjection aging time, had higher (P < .05) sensory tenderness rating than control with the same total aging time (5.2, 5.5, 5.8, and 6.1 vs 4.3, 4.8, 5.1, and 5.3, respectively). Calcium chloride injection at d 14 reduced shear force (.7 kg) and increased tenderness rating (.7 units) as effectively (P > .05) as injection at d 2 (1.2 kg and .8 units, respectively). Calcium chloride-injected steaks had higher (P < .05) juiciness ratings than control steaks. Postrigor calcium chloride injection reduced (P < .05) shear force within 1 d after injection and resulted in more tender meat through 14 d after injection. Extended postinjection aging (35 d) had little effect on color display stability. Calcium-activated tenderization can be applied as late as 14 d postmortem and will reduce the occurrence of tough meat if aging is limited.

Key Words: Beef, Calcium Chloride, Cooking, Injection, Tenderness

Introduction

The original procedures for tenderizing meat with calcium chloride involved infusing lamb carcasses immediately after stunning with 10% of their body weight of a .3 M calcium chloride solution (Koohmaraie et al., 1988, 1989, 1990; Koohmaraie and Shackelford, 1991). Numerous modifications to the original procedures have been made to facilitate commercialization of the process (Wheeler et al., 1991, 1992, 1993, 1994). Currently recommended procedures include injecting cuts of meat at 1 or 2 d postmortem at 5% of cut weight with a .2 M calcium chloride solution and aging an additional 7 d. The efficacy of commercial application (Lansdell et al., 1995) and consumer acceptance (Hoover et al, 1995; Miller et al., 1995) of the calcium-activated tenderization (CAT) process has been established. However, some segments of the meat industry that may want to apply the CAT process do not have access to the meat within the first 2 d postmortem. In addition, there is large variation in the amount of aging time cuts of meat receive before purchase (Morgan et al., 1991). However, the response time for tenderization after postrigor calcium chloride injection has not been demonstrated. Thus, the objectives of this work were to 1) compare the effect of injecting calcium chloride at 2 vs 14 d postmortem, 2) compare the effect of 7 vs 35 d of postinjection aging time, and 3) determine the tenderness response curve in postrigor calcium chloride-treated meat.

Materials and Methods

The Roman L. Hruska U.S. Meat Animal Research Center Animal Care and Use Committee approved the use and treatment of animals in these studies according to guidelines established by the USDA.
Ten 19-mo-old crossbred heifers that had been culled for reproductive failure from either Hereford, Angus, or MARC III (¼ Angus, ¼ Hereford, ¼ Red Poll, ¼ Pinzgauer) dams and Brahman (3), Hereford (3), Boran (2), Angus (1), or Tuli (1) sires were removed from each side at 24 h postmortem. The subprimals from alternating sides were assigned to control or calcium chloride treatment. Treated subprimals were injected (Koch model 12354, Kansas City, MO) at 2 or 14 d postmortem (alternating between ribeye roll and strip loin) with 5% by weight of a 200 mM calcium chloride solution. The injected subprimals were allowed to equilibrate for 5 min then weighed and vacuum-packaged. Alternating halves of each treated subprimal were aged either 7 or 35 d after injection at 1°C. The control subprimals were halved and vacuum-packaged, and the four sections were assigned to 9, 21, 37, or 49 d of aging to provide a matched control with the same total postmortem aging time for each injection treatment. The experiment was conducted as a factorially arranged split-plot design with calcium chloride injection as the whole plot treatment (n = 40) and injection time and postinjection aging time as the factorially arranged split-plot.

After the appropriate aging time (9, 21, 37, or 49 d postmortem, depending on treatment), sections were unpackaged and cut into four 2.54-cm-thick steaks. The first steak was used for Warner-Bratzler shear force measurement, the second and third for trained sensory evaluation, and the fourth steak for assessment of color stability during display. The steaks for shear force and sensory evaluation were vacuum-packaged and frozen at −20°C. For each trait, only the longissimus thoracis et lumbrorum was evaluated. The color evaluation steak was placed in a foam tray, overwrapped with polyvinyl chloride film (O2 transmission of 1,000 mL/645 cm2), and displayed in simulated retail conditions at 1°C under 2,152 lx of ultralume fluorescent light (F40/30U, 30,000K; Philips Lighting, Roselle, IL) continuously for 7 d. The steaks were evaluated for percentage of surface discoloration (1 = 0%, 2 = 1 to 19%, 3 = 20 to 39%, 4 = 40 to 59%, 5 = 60 to 79%, 6 = 80 to 99%, and 7 = 100%) according to AMSA (1991) on d 0, 1, 3, 5, and 7 of display (repeated measures) by a five-member, trained panel. Discoloration was defined as a predominantly brown color. Hunter a* values were obtained on d 0, 1, 3, 5, and 7 of display with a Minolta colorimeter (model CR-200b, Minolta, Ramsey, NJ). Values were obtained by averaging four readings at different locations on the steak’s surface. Because a measure of the proportion of discolored area was obtained by the panel, the Hunter color readings were confined to the nondiscolored area of the steak’s surface to monitor changes in the portion still “red.”

Steaks for Warner-Bratzler shear force and sensory evaluation were thawed to 2°C then cooked on a Farberware model 450N open hearth electric broiler (Farberware, Bronx, NY) to a 70°C internal temperature. The steaks were turned after reaching 40°C. Temperature was monitored with iron constantan thermocouple wires inserted into the geometric center of a steak and attached to a model 205 data logger (Beckman Industrial, San Diego, CA). Warner-Bratzler shear force was conducted as described by Wheeler et al. (1995). Internal cooked color was scored immediately after cooking was completed (1 = very rare, 6 = very well done) according to AMSA (1995). The cooked steaks for shear force were chilled 24 h at 3°C, and then six, 1.27-cm-diameter cores were removed parallel to the muscle fiber orientation. Cores were sheared once each on an Instron Universal Testing Machine model 1135 (Instron, Canton, MA) with a Warner-Bratzler attachment and 5 cm/min crosshead speed.

Cooked steaks for trained sensory evaluation were held in covered glass baking dishes at 70°C for up to 30 min before they were cut into 1 cm × 1 cm × 1 cm thickness cubes and served warm to an eight-member sensory panel trained according to Cross et al. (1978). Each panelist independently evaluated three cubes from each sample for juiciness, tenderness, and beef flavor intensity on 8-point scales (8 = extremely juicy, tender, and intense; 1 = extremely dry, tough, and bland) and off-flavor on a 4-point scale (4 = none, 1 = intense). Scores for each sample were the mean of scores from all eight panelists. Four experimental samples were served in each of two sessions per day, 3 d/wk, for 10 total evaluation days over 3 weeks. In addition, two paired steaks were served each day (one per session) to monitor panel performance. The first session was initiated with a warm-up sample.

**Experiment 2**

Ten 19-mo-old crossbred heifers that had been culled for reproductive failure from either Hereford, Angus, or MARC III (¼ Angus, ¼ Hereford, ¼ Red Poll, ¼ Pinzgauer) dams and Brahman sires were fed a diet consisting of 70% corn, 25% corn silage, and 5% supplement (dry matter basis) for 12 wk. The heifers were slaughtered humanely, and the carcasses were chilled at 0°C for 48 h.

The IMPS #180 strip loin (longissimus lumbrorum) and #112 ribeye roll (longissimus thoracis) were removed from the right sides at 48 h postmortem. Within each animal, one subprimal was assigned to
control and one to calcium chloride treatment. Treated subprimals were injected as described in Exp. 1, vacuum-packaged, and stored at 1°C. At 1 d after injection (3 d postmortem), six 2.54-cm-thick steaks were cut from all subprimals, and all steaks were vacuum-packaged except d-1 steaks. One steak from each subprimal was assigned to 1, 2, 6, 8, 12, or 14 d of postinjection aging with location within the subprimal alternated among animals. At the end of the appropriate aging time, steaks were cooked fresh (never frozen) and sheared as described for Exp. 1.

Statistical Analyses

Shear force, sensory, and cooking data from Exp. 1 were analyzed by ANOVA (SAS, 1988) for a factorially arranged split-plot design with calcium chloride as the whole plot treatment, and injection time (d 2 or 14) and postinjection aging time (7 or 35 d) as the factorially arranged split-plot treatments (Steel and Torrie, 1980). Color data were analyzed as repeated measures. Animal × calcium chloride treatment was the whole plot error term.

Data from Exp. 2 were analyzed by ANOVA for a split-plot design with calcium chloride as the whole plot treatment, animal × calcium chloride treatment the whole plot error term, and postinjection aging time the split-plot treatment (repeated measures). Mean separation for a significant main effect (P < .05) was accomplished with the PDIF option (a pair-wise t-test) of the least squares procedures (SAS, 1988).

Results

Experiment 1

Warner-Bratzler shear force was decreased (P < .05) by calcium chloride injection (Table 1). Furthermore, shear force was lower (P < .05) after 35 d compared to 7 d postinjection aging time in calcium chloride-treated steaks (Table 2). However, injection time did not affect (P > .05) shear force. The two-way interaction of treatment and postinjection aging time was significant (P < .05) for shear force. None of the other interactions was significant, but the mean values for the three-way interaction are shown in Figure 1 to more completely present the results. The interaction of treatment and postinjection aging time was detected for shear force because the difference between control and calcium chloride was greater with 7-d (1.4 kg) aging than with 35-d (.5 kg) aging (Figure 1). Shear force in the control longissimus declined through 37 d postmortem. Calcium chloride treatment with 7-d aging had lower (P < .05) shear force than the control regardless of injection time. However, calcium chloride treatment with 35-d aging was not different (P > .05) from the control. Calcium chloride-injected longissimus at d 2 with 7 d of additional aging tended (P = .09) to have lower shear force than control longissimus aged 21 d.

Percentage cooking loss was higher (P < .05) for calcium chloride-treated steaks (due to the added water) than for the control steaks (Table 1). Calcium chloride injection at 14 d postmortem and 7-d postinjection aging time resulted in greater (P < .05) cooking losses than injection at 2 d and 35-d postinjection aging, respectively (Table 2). Cooked color score was higher (P < .05) for calcium-chloride treated longissimus (more well done) than for control longissimus. Postmortem injection time and postinjection aging time did not affect (P > .05) cooked color score. No interactions were significant (P > .05) for percentage cooking loss or cooked color score. Calcium chloride injection improved (P < .05) tenderness rating (Table 1). Steaks injected on d 14 had a higher tenderness rating (P < .05) than steaks.
Figure 1. Mean Warner-Bratzler shear force values for the three-way interaction of treatment × injection time × postinjection aging time. Values in parentheses are standard deviations. The main effects of treatment and postinjection aging time and the treatment × postinjection aging time interaction were significant (\( P < .05 \)). Control samples were aged either 9, 21, 37, or 49 d. Calcium chloride-treated samples included the following: 2−7, injected with calcium chloride on d 2 postmortem and aged for 7 d after injection; 14−7, injected with calcium chloride on d 14 postmortem and aged for 7 d after injection; 2−35, injected with calcium chloride on d 2 postmortem and aged for 35 d after injection; 14−35, injected with calcium chloride on d 14 postmortem and aged for 35 d after injection. Bars bearing a common superscript are not different (\( P > .05 \)). Pooled SEM = .29.

Table 2. Means for the contrasts of postmortem injection time and postinjection aging time within CaCl₂ treatment on Warner-Bratzler shear force, sensory, and cooking traits of calcium chloride-injected longissimus

<table>
<thead>
<tr>
<th>Trait</th>
<th>Postmortem injection time</th>
<th>Postinjection aging time</th>
<th>Pooled SEM</th>
<th>( P &gt; F )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 d</td>
<td>14 d</td>
<td>7 d</td>
<td>35 d</td>
</tr>
<tr>
<td>n</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Shear force, kg</td>
<td>5.48</td>
<td>5.12</td>
<td>5.97</td>
<td>4.63</td>
</tr>
<tr>
<td>Cooking loss, % a</td>
<td>28.2</td>
<td>29.9</td>
<td>30.3</td>
<td>27.8</td>
</tr>
<tr>
<td>Cooked color score ab</td>
<td>4.4</td>
<td>4.6</td>
<td>4.5</td>
<td>4.6</td>
</tr>
<tr>
<td>Tenderness c</td>
<td>5.48</td>
<td>5.76</td>
<td>5.34</td>
<td>5.90</td>
</tr>
<tr>
<td>Juiciness c</td>
<td>5.71</td>
<td>5.45</td>
<td>5.51</td>
<td>5.65</td>
</tr>
<tr>
<td>Beef flavor intensity c</td>
<td>3.83</td>
<td>3.73</td>
<td>3.96</td>
<td>3.60</td>
</tr>
<tr>
<td>Off-flavor d</td>
<td>2.11</td>
<td>2.02</td>
<td>2.21</td>
<td>1.92</td>
</tr>
<tr>
<td>Cooking loss, % c</td>
<td>33.9</td>
<td>35.0</td>
<td>35.0</td>
<td>34.0</td>
</tr>
<tr>
<td>Cooking time, min (^e)</td>
<td>30.2</td>
<td>30.8</td>
<td>28.6</td>
<td>32.3</td>
</tr>
</tbody>
</table>

\( ^a \)Cooking traits for steaks evaluated for Warner-Bratzler shear force.  
\( ^b \)1 = very rare, 6 = very well done.  
\( ^c \)1 = extremely tough, dry, bland.  
\( ^d \)1 = intense.  
\( ^e \)Cooking traits for steaks evaluated for sensory traits.  
\( ^f \)Postmortem injection time.  
\( ^g \)Postinjection aging time.
then aging for 7 d tended (P = .07) to improve tenderness rating over controls aged 21 d.

Juiciness ratings were higher (P < .05) in calcium chloride-treated than in control steaks for d-2 than for d-14 injection time and for 35-d than for 7-d postinjection aging time (Table 1). This indicates that after purge during aging and losses during cooking, enough added water was retained to improve juiciness. Beef flavor intensity ratings were not affected (P > .05) by injection time (Table 2). Control steaks had higher (P < .05) beef flavor intensity ratings than calcium chloride-treated steaks (Table 1), and 7-d postinjection aging time increased (P < .05) beef flavor intensity ratings compared to 35-d postinjection aging time (Table 2). Injecting calcium chloride, a later injection time, and greater postinjection aging time all resulted in greater (P < .05) off-flavors.

Discoloration scores were affected (P < .05) by treatment, display time, and the treatment × display time interaction. Discoloration scores of steaks in simulated retail display at 1°C indicated no discoloration (P > .05) through 5 d of display regardless of calcium chloride or injection time treatment (Figure 3). Only data for 35-d postinjection aging time were shown because data from 7-d postinjection aging time were lost due to elevated temperature in the display cooler. After 7 d of display, control steaks aged 37 d still had no discoloration. However, steaks injected with calcium chloride on d 2 and aged 35 d postinjection and control steaks aged 49 d had slightly greater (P < .05) discoloration. The most discoloration after 7 d of display was in the calcium chloride-treated steaks injected on d 14 and aged 35 d postinjection, although these steaks had less than 1% discoloration.

Hunter a* values were affected (P < .05) by treatment, display time, and the injection time × display time interaction (Figure 4). Hunter a* values declined (P < .05) as display time increased. Treatment did not affect (P > .05) a* values at 0 or 1 d of display time. After 3 d of display, neither calcium chloride treatment nor injection time affected (P > .05) a* values. However, calcium chloride-treated steaks injected on d 14 with 35-d postinjection aging time had lower (P < .05) a* values than control steaks with 37-d aging. After 5 d of display, injection time did not affect (P > .05) a* values, but steaks injected at 14 d postmortem had lower (P < .05) a* values than control steaks with either 37- or 49-d aging. After 7 d of display, calcium chloride-injected steaks had lower (P < .05) a* values than their respective control steaks with the same total aging time. However, injection time did not affect (P > .05) a* values.

**Experiment 2**

Within 1 d after calcium chloride injection, treated steaks were more tender than control steaks (Figure 5). Thereafter, the aging response of control and calcium chloride-treated steaks was approximately parallel; treated steaks remained more tender (P < .05) than control steaks at all aging times except 8 d postinjection. Neither control nor treated steaks declined further (P > .05) in shear force after 6 d of postinjection aging. Calcium chloride-treated steaks were as tender after 1-d postinjection aging as the control steaks after 14-d aging. In addition, based on standard deviations in Figures 1 and 2, but not Figure 5, the amount of variation in tenderness or shear force was not affected by calcium chloride injection.
IMPROVING MEAT TENDERNESS WITH CALCIUM

Figure 3. Mean steak surface discoloration scores for the three-way interaction of treatment \( \times \) postmortem injection time \( \times \) display time during simulated retail display at 1°C. The main effects of treatment (control or calcium chloride), display time (0, 1, 3, 5, 7 d), and the interaction of treatment \( \times \) display time were significant \((P < .05)\). Control samples were aged for either 37 or 49 d. Calcium chloride-treated samples included the following: 2−35, injected with calcium chloride on d 2 postmortem and aged for 35 d after injection; 14−35, injected with calcium chloride on d 14 postmortem and aged for 35 d after injection. The data for the 7-d postinjection aging time treatments and their corresponding controls were lost due to elevation of display cooler temperature. Bars bearing a common superscript are not different \((P > .05)\). Error bars are pooled SEM.

In Exp. 2, shear force was not reduced \((P > .05)\) with additional aging past 6 d postinjection. However, in Exp. 1, shear force was reduced \((P < .05)\) with 28 d of additional aging (from 7 to 35 d postinjection) after injecting at 2 d postmortem. This apparent difference in response to calcium-activated tenderization can be explained by the initial tenderness of the samples from their respective experiments. In Exp. 1, the control longissimus at 9 d postmortem had a shear force of 7.2 kg. In Exp. 2, the control longissimus at 8

Figure 4. Mean Hunter a* values for the three-way interaction of treatment \( \times \) postmortem injection time \( \times \) display time during simulated retail display at 1°C. The main effects of treatment (control or calcium chloride), postmortem injection time (2 or 14 d postmortem), display time (0, 1, 3, 5, 7 d), and the interaction of injection time \( \times \) display time were significant \((P < .05)\). See Figure 3 legend for treatment details. Bars bearing a common superscript are not different \((P > .05)\). Error bars are pooled SEM.
Figure 5. Mean Warner-Bratzler shear force values for the two-way interaction of calcium chloride treatment × postinjection aging time. Values in parentheses are standard deviations. The main effects of treatment and postinjection aging time were significant (P < .05). All samples were aged for either 1, 2, 6, 8, 12, or 14 d after injection. Means bearing a common superscript are not different (P > .05). Pooled SEM = .26.

d postmortem (6 d postinjection) had a shear force of 5.6 kg. Thus, the rate and magnitude of the tenderization response from postrigor calcium chloride injection depends on the initial tenderness of the meat.

Discussion

Some industry segments that might apply calcium-activated tenderization technology do not have access to the meat by 2 d postmortem. These include purveyors, retailers, restaurants, and small, branded meat companies that buy subprimals or contract their slaughter. This study establishes that calcium chloride can be injected as late as 14 d postmortem and be as effective for tenderizing meat as injection on d 1 or 2 postmortem. This effect is possible because the mechanism of tenderization is through activation of the calpain enzymes (Koohmaraie et al., 1988). Because m-calpain activity does not decline during postmortem storage (Koohmaraie et al., 1987), it is available for activation at any time postmortem. Thus, calcium-activated tenderization potentially could be initiated at any time postmortem.

Application of calcium-activated tenderization to prerigor muscle immediately postmortem results in rapid tenderization that is complete within 1 d postmortem (Koohmaraie et al., 1988, 1989; Koohmaraie and Shackelford, 1991; Wheeler et al., 1991). However, it was hypothesized that postrigor application would result in a much decreased rate of tenderization due to the lower pH and temperature of the meat. For this reason, experiments using postrigor application of calcium-activated tenderization utilized at least 7 d of postinjection aging to allow time for the tenderization to take place (Wheeler et al., 1992, 1993; Kerth et al., 1995; Lansdell et al., 1995; Miller et al., 1995; Wulf et al., 1996). However, the complete tenderness response curve for postrigor calcium chloride injection has never been documented. Data from Exp. 2 indicate that longissimus with 1-d postinjection aging time was as tender as the control after 14 d of postinjection aging (16 d postmortem), although calcium-tenderized meat continued to improve in tenderness with increased aging. This result does not imply that calcium-tenderized meat should not be aged. However, these data indicate that the calcium-activated tenderization process would reduce potential meat toughness problems in meat that receives limited aging time.

The longest postinjection aging time reported in the literature for calcium chloride treatment is 14 d (Diles et al., 1994; Kerth et al., 1995). Kerth et al. (1995) injected beef longissimus from A-maturity carcasses with .2 M calcium chloride at 5%. They reported that 14-d compared to 7-d postinjection aging resulted in decreased shear force, increased purge, no affect on sensory traits, and no difference relative to the control.
for discoloration, Hunter a* value, or browning during retail display. Diles et al. (1994) injected beef longissimus from cow carcasses (> 8 yr of age) with .2 M calcium chloride at 10% and compared 7- vs 14-d postinjection aging. They reported that 14-d aging resulted in decreased shear force, increased tenderness and flavor intensity rating, and decreased purge relative to 7-d postinjection aging.

Due to the oxidative properties of salts (Chang and Watts, 1950), including calcium chloride, and the large variation in duration of aging before beef cuts are purchased at retail (Morgan et al., 1991), we were interested in the potential detrimental effects of extended aging (35 vs 7 d) on beef flavor and color stability. Data from Exp. 1 indicate that extended aging, which is likely to occur during the normal distribution of the product, results in additional tenderization with minimal effects on subsequent color display stability through 3 d of display. The longissimus has greater color stability during display than most other muscles (Faustman and Cassens, 1990); this explains why calcium chloride had less effect on color stability of longissimus during display even after extended aging (Figure 3) than was reported by Wheeler et al. (1996) for biceps femoris. However, extended aging was associated with decreased beef flavor intensity and increased off-flavors. This result may have been partially due to the use of non-grain-fed beef.

Some studies (the present one and Wheeler et al., 1993) have reported that trained sensory panels detected decreased beef flavor intensity and increased off-flavors in calcium chloride treated meat (at 5% injection of .2 M calcium chloride), whereas other studies have not detected those differences (Kerth et al., 1995). However, consumer studies of the calcium-activated tenderization process indicate that tenderness and flavor are improved over those of control steaks (Hoover et al., 1995; Miller et al., 1995) and have never indicated a flavor problem associated with calcium-activated tenderization. Trained sensory panels evaluate meat cooked without seasonings; thus, any added ingredient with any flavor can usually be detected. Calcium chloride has a metallic, bitter flavor; thus, it is not surprising that trained sensory panels can detect it in meat. However, it is more relevant that consumer evaluation of calcium-activated tenderization using steaks seasoned normally indicated that flavor was improved slightly (probably due to the additional saltiness imparted by calcium chloride) relative to flavor of control steaks. Thus, we recommend at least 7 d, but not more than 35 d, of aging time after calcium chloride injection.

Implications

Calcium-activated tenderization may be applied up to 14 d postmortem to improve meat tenderness. Calcium-activated tenderization could be used to reduce tenderness problems in meat with limited or extended aging time.

Literature Cited