

# The Effect of Postmortem Time of Injection and Freezing on the Effectiveness of Calcium Chloride for Improving Beef Tenderness<sup>1,2</sup>

T. L. Wheeler, J. D. Crouse<sup>3</sup>, and M. Koohmaraie

Roman L. Hruska U.S. Meat Animal Research Center, ARS, USDA, Clay Center, NE 68933-0166

**ABSTRACT:** Three experiments were conducted to determine the effect of freezing and time postmortem on the effectiveness of injecting CaCl<sub>2</sub> to tenderize beef. In Exp. 1, longissimus muscle treatments included 1) control 0 h, 2) CaCl<sub>2</sub>-injected 0 h, 3) control 24 h, and 4) CaCl<sub>2</sub>-injected 24 h. Injection consisted of .3 M CaCl<sub>2</sub> at 10% by weight. Injecting CaCl<sub>2</sub> at 24 h postmortem reduced ( $P < .05$ ) shear force requirements compared with the 24 h control but did not ( $P < .05$ ) tenderize meat as much as injecting at 0 h. In Exp. 2, longissimus muscle treatments included the following: 1) aged 2 d; 2) aged 7 d; 3) frozen d 1, thawed, aged 6 d; 4) CaCl<sub>2</sub>-injected d 1, aged 6 d; 5) frozen d 1, thawed, CaCl<sub>2</sub>-injected, aged 6 d; and 6) CaCl<sub>2</sub>-injected d 1, frozen, thawed, aged 6 d. Injection alone at d 1 or freezing, then thawing and injecting resulted in the lowest ( $P < .05$ ) shear

force requirements. In Exp. 3, longissimus muscle treatments included the following: 1) aged 1 d; 2) aged 7 d; 3) CaCl<sub>2</sub>-injected 0 h, aged 7 d; 4) CaCl<sub>2</sub>-injected d 1, aged 6 d; 5) frozen d 1, thawed, aged 6 d; and 6) frozen, thawed, CaCl<sub>2</sub>-injected, aged 6 d. Both d-1 injection alone and freezing, thawing, then injecting resulted in meat with shear force requirements similar to those of 0-h injected meat. The effect of treatments on cooking loss was inconsistent. Treatments that reduced shear force also reduced ( $P < .05$ ) calpain and calpastatin activity proportionately. Calcium chloride injection at 24 h postmortem tenderizes meat as effectively as injection at 0 h. This process provides the beef industry with the technology needed to produce consistently tender meat from virtually all meat sources.

Key Words: Beef, Calcium Chloride, Calpain, Freezing, Tenderness

J. Anim. Sci. 1992. 70:3451-3457

## Introduction

The National Beef Tenderness Survey (Morgan et al., 1991b) revealed that current beef production practices result in considerable variation in meat tenderness and an unacceptable percentage of tough meat. One potential solution to this problem

uses calcium chloride, either infused into the carcass or injected directly into the muscle soon after exsanguination, to enhance and accelerate the postmortem tenderization process in both ovine and bovine muscle (Koohmaraie et al., 1988, 1989, 1990; Morgan et al., 1991a; Wheeler et al., 1991).

If this technology could be applied after 24 h postmortem it would avoid conflict with inspection and grading procedures. Evidence that this would be possible was provided by soaking (Alarcon-Rojo and Dransfield, 1989) or marinating (Whipple and Koohmaraie, 1992a) postrigor muscle in a calcium chloride solution.

The objective of this research was to determine whether injecting calcium chloride into postrigor meat tenderizes the meat to the same extent that it tenderizes prerigor meat and to determine whether additional tenderization results from freezing and thawing meat before or after injecting it with calcium chloride.

<sup>1</sup>The authors express their gratitude to P. Ekeren, K. Theer, and P. Tammen for their technical assistance in the execution of these experiments and to C. Grummert for secretarial assistance.

<sup>2</sup>Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

<sup>3</sup>Current address: USDA-ARS-NPA, 1201 Oakridge Dr., Suite 150, Ft. Collins, CO 80525.

Received April 10, 1992.

Accepted July 13, 1992.

## Materials and Methods

**Animals.** The Roman L. Hruska U.S. Meat Animal Research Center Animal Care and Use Committee approved the use of animals in this study. Experiment 1 used 11 *Bos taurus* crossbred (Hereford, Angus, and Pinzgauer) bulls. Experiments 2 and 3 each used seven *Bos indicus* crossbred (3/8, 1/2, or 5/8 Brahman or Sahiwal) bulls. All animals were fed the experimental diet from 8 mo of age until they were slaughtered at 16 mo of age. Animals were allowed ad libitum access to a diet formulated to meet NRC requirements for growing beef cattle (NRC, 1984). The diet consisted of 76% corn silage, 20% wet corn, 2% soybean meal, and 2% supplement (supplement composition: 54% soybean meal, 17.9% corn, 17.8% limestone, 6% dicalcium carbonate, 3% urea, .6% vitamins A, D, and E, .4% Rumensin 60, and 1% sulfur) with 11.7% CP and 2.83 Mcal of ME/kg of feed as fed. All animals were slaughtered according to standard procedures at the Roman L. Hruska U.S. Meat Animal Research Center. The longissimus muscle from the 9th thoracic to the 3rd lumbar vertebrae was removed from the left side of the carcasses in all experiments.

**Experiment 1.** Within 30 min after exsanguination, the longissimus muscle was cut into four equal sections that were assigned to one of four treatments: 1) vacuum-packaged immediately (0 h) and then tumbled; 2) injected at 10% by weight with a .3 M CaCl<sub>2</sub> solution immediately (0 h), then vacuum-packaged and tumbled; 3) vacuum-packaged and stored at 2°C for 24 h, then tumbled; or 4) vacuum-packaged and stored at 2°C for 24 h, then injected at 10% by weight with a .3 M CaCl<sub>2</sub> solution, vacuum-packaged, and tumbled. The sections were injected with a hand-held stitch needle (four needles) through the transverse surfaces (three insertion positions were used to improve distribution). Sections were vacuum-packaged with a chamber vacuum packaging machine in an oxygen-impermeable film. After tumbling (continuously for 15 min without vacuum in a 35-cm long × 18-cm diameter cylinder), the sections were aged at 2°C until 48 h postmortem then cut into two 2.54-cm-thick steaks. One steak was cooked for Warner-Bratzler shear force measurement on d 2. The other was vacuum-packaged again, stored at 2°C until 5 d postmortem, and then cooked for Warner-Bratzler shear force evaluation.

**Experiment 2.** At 24 h postmortem, the longissimus muscle was cut into six equal sections that were assigned to one of six treatments: 1) aged 2 d; 2) aged 7 d; 3) frozen d 1, thawed, aged 6 d; 4) injected d 1, aged 6 d; 5) frozen d 1, thawed, injected, aged 6 d; or 6) injected d 1, frozen, thawed, aged 6 d. Aging was performed at 2°C and

frozen treatments were frozen at -30°C for 7 d, then thawed at 2°C for 24 h. Injected treatments were injected at 10% by weight with .3 M CaCl<sub>2</sub> with a hand-held stitch needle as described for Exp. 1, except that the needles were inserted through the lateral and medial sides of the sections rather than through the transverse surfaces. All treatments were vacuum-packaged before freezing or aging. At the end of their respective aging times, a 2.54-cm-thick steak was removed from the muscle sections and cooked for Warner-Bratzler shear force determination.

**Experiment 3.** Within 30 min after exsanguination, the longissimus muscle was cut into six equal sections that were assigned to one of six treatments: 1) aged 2 d; 2) aged 7 d; 3) injected immediately, aged 7 d; 4) injected d 1, aged 6 d; 5) frozen d 1, thawed, aged 6 d; or 6) frozen d 1, thawed, injected, aged 6 d. Aging, injecting, freezing, thawing, and cooking procedures were the same as described for Exp. 2. In addition, 5-g muscle samples were obtained 1 d after application of each treatment (for controls: at 1 d postmortem; 0-h inject: at 1 d postmortem; 1 d inject: at 2 d postmortem; frozen: after thawing; freeze/inject: 1 d after thawing and injecting) for determining the activities of  $\mu$ - and m-calpain and calpastatin.

**Cooking and Warner-Bratzler Shear Procedures.** Steaks were broiled on Farberware Open Hearth Electric broilers (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 the steak and attached to a Honeywell potentiometer multipoint recorder (Honeywell, Scarborough, ON, Canada). Steaks were weighed before and after cooking to determine cooking loss. After cooking, the steaks were chilled 24 h at 3°C (Exp. 1 and 3) or 3 h at 3°C (Exp. 2), then six 1.27-cm-diameter cores were removed parallel to the muscle fiber orientation and sheared once each on an Instron model 1132/Microcon II instrument (Instron, Canton, MA) with a Warner-Bratzler shear attachment. The cross-head speed was 5 cm/min.

**Calpain and Calpastatin Activity Determination.** Activities of  $\mu$ -calpain, m-calpain, and calpastatin were determined according to procedures described by Koohmaraie (1990b) with the following modifications. Five-gram muscle samples were extracted. The ionic strength of the supernatants was reduced before ion-exchange chromatography by dialyzing for 20 h against 20 mM Tris, 5 mM EDTA, 7 mM  $\beta$ -mercaptoethanol, pH 7.5, rather than by adding water.

**Statistical Analyses.** Data from Exp. 1 were analyzed by analysis of variance with the GLM procedure of SAS (1989) for a 2 (postmortem time) ×

2 (CaCl<sub>2</sub> treatment) factorial arrangement of an unbalanced, randomized complete block design. The blocking factor was vertical location within the longissimus muscle. Data from Exp. 2 were analyzed by analysis of variance with the GLM procedure of SAS (1989) for an unbalanced, randomized complete block design. The blocking factor was vertical location within the longissimus muscle. Means separation for significant ( $P < .05$ ) main effects was accomplished by Tukey's mean separation test. Data from Exp. 3 were analyzed by analysis of variance with the GLM procedure of SAS (1989) for a completely randomized design. Means separation for a significant ( $P < .05$ ) main effect was accomplished with the PDIF option of the least squares procedures (a pairwise  $t$ -test).

## Results

Animal and carcass traits are shown in Table 1 for characterization purposes. Warner-Bratzler shear force measurements indicated that control meat used in Exp. 1 was relatively tough after 2 and 5 d of postmortem aging (Table 2). Meat that had been injected with calcium chloride at 30 min postmortem had markedly reduced ( $P < .05$ ) shear force requirements at both 2 and 5 d postmortem. However, injecting calcium chloride into postrigor meat (24 h postmortem) resulted in a much smaller reduction ( $P < .05$ ) in shear force than injecting with calcium chloride at 30 min postmortem. Cooking loss was lower ( $P < .05$ ) at 2 d postmortem in meat injected with calcium at 30 min postmortem. However, by 5 d postmortem, both 30-min postmortem treatments had similar ( $P > .05$ ) cooking losses.

In Exp. 2, an attempt was made to enhance the effects of calcium chloride injection into postrigor meat by freezing the meat, both before and after injection (Table 3). In this experiment, injecting calcium chloride at 1 d postmortem, or freezing, then thawing and injecting the meat resulted in a

Table 1. Means and SEM for animal and carcass traits

Trait	Experiment		
	1 (n = 11)	2 (n = 7)	3 (n = 7)
Live wt, kg	415.8 (29.0)	558.6 (24.2)	578.9 (26.1)
Hot carcass wt, kg	244.8 (8.7)	335.2 (13.4)	352.6 (18.9)
Maturity <sup>a</sup>	137.0 (16.0)	165.0 (11.0)	163.0 (12.5)
Marbling degree <sup>b</sup>	292.0 (25.4)	319.0 (23.2)	364.0 (29.9)
USDA quality grade <sup>c</sup>	3.1 (1.4)	3.9 (.7)	4.6 (.5)
Fat thickness, mm	5.0 (1.5)	8.3 (1.2)	9.6 (1.1)
Adjusted fat thickness, mm	4.4 (.8)	7.8 (.2)	9.4 (1.9)
Longissimus muscle area, cm <sup>2</sup>	73.3 (2.9)	84.3 (3.0)	86.2 (5.5)
Kidney, pelvic and heart fat, %	1.5 (.2)	1.7 (.4)	2.6 (.8)
USDA yield grade	1.7 (.2)	2.2 (.1)	2.7 (.4)
Heat ring <sup>d</sup>	2.2 (.3)	1.7 (.2)	1.9 (.9)

<sup>a</sup>100 = A<sup>00</sup>, 200 = B<sup>00</sup>.

<sup>b</sup>200 = Traces<sup>00</sup>, 300 = Slight<sup>00</sup>.

<sup>c</sup>3 = High Standard, 4 = Low Select.

<sup>d</sup>1 = None, 7 = Severe.

level of shear force at 7 d postmortem similar to that usually obtained by injecting immediately after slaughter. Injecting calcium chloride, then freezing, or freezing alone did not ( $P > .05$ ) result in meat with reduced shear force compared to the control aged 7 d. The effect on tenderness of the 1-d injection treatment from Exp. 2 is in contrast to the results of Exp. 1. This discrepancy may partially have been due to the inherently lower shear force requirements of the control meat in Exp. 2 and/or greater retention of the injected solution due to injection method (see Materials and Methods). Cooking loss of the injected treatments was higher than in the controls and cooking rate was not affected, as reported in Exp. 1.

Experiment 3 was conducted to confirm that injecting postrigor and prerigor meat with calcium chloride results in similar tenderization. In addi-

Table 2. Means and SEM for Warner-Bratzler shear (WBS) force and cooking loss for Experiment 1

Trait	Injected 30 min postmortem		Injected 24 h postmortem	
	Control	CaCl <sub>2</sub>	Control	CaCl <sub>2</sub>
WBS, kg				
2 d	10.31 <sup>ab</sup> (.62)	2.95 <sup>c</sup> (.36)	11.14 <sup>a</sup> (.36)	8.43 <sup>b</sup> (.67)
5 d	9.95 <sup>ab</sup> (.64)	3.35 <sup>c</sup> (.37)	11.49 <sup>a</sup> (.67)	8.61 <sup>b</sup> (.72)
Cooking loss, %				
2 d	35.8 <sup>a</sup> (1.4)	28.2 <sup>b</sup> (1.2)	34.2 <sup>a</sup> (1.9)	38.1 <sup>a</sup> (1.1)
5 d	31.7 <sup>bc</sup> (1.6)	29.3 <sup>c</sup> (1.2)	35.3 <sup>ab</sup> (.8)	38.1 <sup>a</sup> (1.5)

<sup>a,b,c</sup>Means in a row lacking a common superscript letter differ ( $P < .05$ ).

Table 3. Means and SEM for Warner-Bratzler shear (WBS) force and cooking loss for Experiment 2

Treatment <sup>a</sup>	Total aging time, d	WBS, kg	Cooking loss, %
Control	2	6.99 <sup>b</sup> (.36)	29.3 <sup>b</sup> (1.1)
Control	7	5.9 <sup>bc</sup> (.62)	23.2 <sup>d</sup> (.9)
Inject 1 d	7	3.69 <sup>de</sup> (.8)	29.0 <sup>bc</sup> (.8)
Freeze	7	4.6 <sup>cd</sup> (.43)	24.2 <sup>cd</sup> (.9)
Inject 1 d/freeze	7	5.18 <sup>c</sup> (.61)	30.6 <sup>b</sup> (1.2)
Freeze/inject	7	3.16 <sup>e</sup> (.43)	31.9 <sup>b</sup> (.7)

<sup>a</sup>See Materials and Methods for details of treatment.

<sup>b,c,d,e</sup>Means within a column lacking a common superscript letter differ ( $P < .05$ ).

tion, activities of the components of the calpain proteolytic system were measured to provide information regarding why some treatments resulted in greater tenderization than others. Meat from the control treatment with 1 or 7 d postmortem aging had greater ( $P < .05$ ) shear force than other treatments (Table 4). Freezing induced an intermediate reduction in shear force. Injecting calcium chloride immediately after slaughter or at 1 d postmortem, with or without prior freezing, resulted in similar and large reductions ( $P < .05$ ) in shear force requirements at 7 d postmortem. Thus, freezing and thawing postrigor meat before injecting it with calcium chloride was not necessary to obtain tenderization similar to that obtained by injecting prerigor meat. Cooking loss was higher ( $P < .05$ ) in meat that was either injected or frozen at 1 d postmortem.

For each treatment, the activities of the components of the calpain proteolytic system were measured 1 d after the application of each particular treatment (see Materials and Methods for details). This protocol was followed to assess the effect of the treatments on the proteinase system

and the subsequent effect on the aging of meat from that treatment. The control and frozen treatments had the most ( $P < .05$ ) residual calpain and calpastatin activity remaining after application of the treatment (Table 4).

## Discussion

The major and rapid tenderization by calcium chloride injection or infusion into prerigor muscle has been well established (Koochmaraie et al., 1988, 1989, 1990; Morgan et al., 1991a; Wheeler et al., 1991), such that essentially all potential postmortem tenderization is accomplished within 24 h postmortem. The extent of tenderization from 0-h injection in Exp. 1 and 3 was consistent with previous results from prerigor infused or injected meat. Shear force requirements have been consistently reduced to 3 to 4 kg in experiments using the following muscles: longissimus muscle from slaughter lambs (Koochmaraie et al., 1988, 1989), *Bos indicus* steers (Koochmaraie et al., 1990), longissimus muscle from sheep fed  $\beta$ -adrenergic agonist (Koochmaraie and Shackelford, 1991), semimembranosus and biceps femoris muscles from *Bos indicus* bulls and late-castrate steers (Wheeler et al., 1991), and longissimus and gluteus medius muscles from 12-yr-old cows (Morgan et al., 1991a). The only exception has been that the shear force requirements of semimembranosus muscles from 12-yr-old cows only declined to 6 kg after calcium chloride injection due to greater background connective tissue in that muscle from that age of animal (Morgan et al., 1991a). In addition, this process would never over-tenderize (such as sometimes occurred with the enzyme papain) due to the self-limiting process of autolysis (Koochmaraie et al., 1987; Koochmaraie, 1992). These data indicate

Table 4. Least squares means for Warner-Bratzler shear (WBS) force, cooking loss, and activities of the calpain proteolytic system for Experiment 3

Treatment <sup>a</sup>	Total aging time, d	WBS, kg	Cooking loss, %	$\mu$ -Calpain <sup>e</sup>	m-Calpain <sup>e</sup>	Calpastatin <sup>f</sup>
Control	1	8.36 <sup>b</sup>	21.9 <sup>c</sup>	—	—	—
Control	7	7.24 <sup>b</sup>	22.6 <sup>c</sup>	.71 <sup>b</sup>	.86 <sup>b</sup>	1.20 <sup>b</sup>
0 h Inject	7	2.81 <sup>d</sup>	21.8 <sup>c</sup>	ND <sup>d</sup>	.14 <sup>d</sup>	.19 <sup>d</sup>
1 d Inject	7	3.85 <sup>d</sup>	27.1 <sup>b</sup>	.18 <sup>d</sup>	.45 <sup>c</sup>	.69 <sup>c</sup>
Freeze	7	5.13 <sup>c</sup>	27.8 <sup>b</sup>	.35 <sup>c</sup>	.67 <sup>bc</sup>	1.14 <sup>b</sup>
Freeze/inject	7	2.56 <sup>d</sup>	30.4 <sup>b</sup>	.05 <sup>d</sup>	.30 <sup>cd</sup>	.33 <sup>d</sup>
SEM	—	.46	1.4	.06	.10	.11

<sup>a</sup>See Materials and Methods for details of treatments.

<sup>b,c,d</sup>Means within a column lacking a common superscript letter differ ( $P < .05$ ). ND = Not detectable.

<sup>e</sup>Total activity per gram of muscle (caseinolytic activity).

<sup>f</sup>Inhibition of casein hydrolysis by m-calpain per gram of muscle.

the potential for ensuring that virtually all meat is very tender, regardless of the source. In addition, calcium chloride has been approved by FDA as GRAS (Generally Recognized as Safe) at maximum levels of 3% of an .8 M solution (reg. #318.7 [c] [4]; FSIS, 1973). However, the adoption of this technology by the meat industry may be hampered by the need to introduce the exogenous calcium to prerigor muscle due to inspection problems. It would logistically be much easier to inject a calcium chloride solution into meat after 24 h postmortem. Evidence that this may be possible was obtained from data showing improved tenderness from soaking 1 d postmortem beef strips in 30 mM calcium chloride for 24 h (Alarcon-Rojo and Dransfield, 1989) or marinating 5-d postmortem steaks in 150 mM calcium chloride for 48 h (Whipple and Koohmaraie, 1992a).

Based on previous data (Koohmaraie et al., 1988, 1989, 1990; Alarcon-Rojo and Dransfield, 1989; Koohmaraie and Shackelford, 1991), it seems the mode of action of calcium chloride-induced tenderization is through proteolysis from activation of the calpain proteinases. During normal postmortem aging, the intracellular calcium concentration reaches a level (~ 100  $\mu$ M) high enough to activate only  $\mu$ -calpain and, thus, m-calpain activity remains intact in postmortem muscle (Vidalenc et al., 1983; Koohmaraie et al., 1987). Addition of sufficient exogenous calcium to prerigor muscle activates both  $\mu$ - and m-calpain, resulting in greater and more rapid tenderization than would occur without the additional calcium. Theoretically, the addition of exogenous calcium to postrigor meat should result in tenderization similar to that obtained by adding calcium to prerigor meat, albeit the tenderization process would occur more slowly than in prerigor meat due to lower temperature and pH (Koohmaraie et al., 1986). This theory is based on the fact that, because m-calpain activity is not affected by postmortem aging, it should be available for activation. However, it is possible that changes in cell membrane permeability during rigor development could restrict the entry of exogenous calcium into the muscle cell and, thus, prevent the complete activation of m-calpain. If this were the case, some additional means of ensuring sufficient calcium enters the cell to activate m-calpain would be needed.

Muscle cell membrane disruption caused by freezing (Bevilacqua et al., 1979; Añón and Calvelo, 1980) could facilitate calcium uptake by the cell and, thus, enhance calpain activation. Furthermore, it has been shown that frozen storage of muscle decreases calpastatin activity (Koohmaraie, 1990b; Whipple and Koohmaraie, 1992b) and enhances tenderization during subsequent thawing and aging (Crouse and Kooh-

maraie, 1990). Thus, injecting previously frozen and thawed meat with calcium chloride may enhance tenderization by increasing calpain activity through increasing access to calcium and/or reducing calpastatin activity (Whipple and Koohmaraie, 1992b).

Results from Exp. 1 seemed to indicate that calcium did not enter the postrigor muscle cell in sufficient quantity to fully activate m-calpain. The reduction in shear force of postrigor injected meat was substantially less than that of prerigor injected meat. However, we observed while conducting the experiment that the calcium chloride solution did not absorb as readily into the postrigor meat as it did into the prerigor meat. The injection procedure (injecting the longissimus muscle sections through the transverse surfaces, which produced needle holes essentially parallel to fiber direction) may have contributed to the decreased retention of the injection solution. Subsequently, in Exp. 2 and 3, the injection sites were through the lateral and medial sides of the meat sections, which resulted in much greater retention of injected solution. Thus, in Exp. 2, although a prerigor injected treatment was not included, the postrigor injected treatment had a shear force (3.69 kg) similar to that usually obtained from prerigor injection. Freezing, then thawing and injecting with calcium chloride did not result in significantly lower shear force than injecting alone. Thus, apparently enough calcium was able to enter the postrigor muscle cell to activate m-calpain and freezing was not necessary. However, because of the intermediate level of shear force for the control meat, the ability of postrigor injection to tenderize tougher meat sufficiently remained in doubt.

In Exp. 3, the control meat was less tender than that in Exp. 2. In addition, the prerigor injection treatment was included for comparison. This experiment confirmed that postrigor calcium chloride injection tenderizes meat as well as prerigor injection. Again, as in Exp. 2, the freeze then thaw and inject treatment did not result in more tender meat than d-1 injection alone. However, Whipple and Koohmaraie (1992b) reported greater reduction in shear force if steaks had been frozen and thawed before calcium chloride marination than if they were marinated alone. This difference could be due to differences in calcium concentration and application, postmortem time of treatment, or length of frozen storage. These data indicate that either pre- or postrigor injection of calcium chloride could be used to improve meat tenderness, depending on individual operating conditions. Furthermore, prerigor injection could be used in conjunction with hot-boning (Wheeler et al., 1991) to reduce processing time and costs.

Cooking traits in Exp. 1 are not in agreement with the results of Wheeler et al. (1991). They reported greater cooking loss with calcium-injected meat. However, Koohmaraie et al. (1990) reported similar (ovine) or increased (bovine) cooking loss with calcium injection of prerigor meat. Experiment 3 indicates that postrigor injection, freezing, or the combination, resulted in greater cooking losses. However, results of Exp. 2 were less conclusive.

The remaining  $\mu$ - and m-calpain activity measured after treatment applications indicated the extent of activation of the proteinases. The greater activities of the calpains indicated that they had been less activated and, thus, less autolysis had occurred (Koohmaraie et al., 1987, 1989). This also signified that less proteolysis of myofibrillar proteins had occurred, which resulted in meat that was less tender. In addition, the remaining calpastatin activity indicated the relative potential inhibition of calpain activity that remained. The freezing treatment alone did not reduce calpastatin activity, probably due to the short frozen storage time (7 d). Koohmaraie (1990b) reported a 33% decline in calpastatin activity after 2 wk of frozen storage. As expected, the differences in activities of the components of the calpain proteolytic system closely reflected differences in shear force values. The control and frozen treatments had the most residual calpain and calpastatin activity, which corresponds with the higher shear force values for meat from those treatments. A comparison of the activities of the calpain system between 0-h inject and 1-d inject treatments indicates that the exogenous calcium did enter the postrigor muscle cell, although to a lesser extent than in the prerigor muscle. These data indicate that any additional tenderization from calcium injection of previously frozen meat (frozen 1 wk) was due to greater calpain activation, not to decreased calpastatin activity. These results were consistent with other data indicating that the remaining activities of calpain and calpastatin at various postmortem times reflect the extent of tenderization (Koohmaraie et al., 1988; Koohmaraie 1990a; Whipple et al., 1990).

Industry adoption of calcium chloride injection technology will depend on its effect on meat characteristics in addition to tenderness, such as flavor, shelf-life, and retail lean color. Trained flavor profile analysis of lamb meat from carcasses infused at 10% with .3 M calcium chloride detected no effect on desirable flavor attributes and only slightly increased salty and bitter flavor notes (St. Angelo et al., 1991). However, Morgan et al. (1991a) reported increased livery, salty, and bitter flavor notes with injection at 10% of .3 M calcium chloride. It is anticipated that industry

application of this process would use a lower concentration of calcium chloride that would avoid potential flavor problems, but maintain the desired increase in tenderness by 7 d postmortem. This possibility (as well as the effects on shelf-life and color) is currently under investigation.

## Implications

Injecting a calcium chloride solution into postrigor muscle tenderizes the meat as well as injecting prerigor meat. Freezing the meat first, then thawing and injecting did not provide additional tenderization. Very tender meat can be produced from almost all meat sources by injecting either prerigor or postrigor meat with calcium chloride, depending on which process is better suited to specific operating conditions.

## Literature Cited

- Alarcon-Rojo, A., and E. Dransfield. 1989. Effect of calcium ions on texture of beef during conditioning. *Proc. Int. Congr. Meat Sci. Technol. (Copenhagen)* 35:1141.
- Añón, M. C., and A. Calvelo. 1980. Freezing rate effects on the drip loss of frozen beef. *Meat Sci.* 4:1.
- Bevilacqua, A., N. E. Zaritzky, and A. Calvelo. 1979. Histological measurements of ice in frozen beef. *J. Food Technol.* 14:237.
- Crouse, J. D., and M. Koohmaraie. 1990. Effect of freezing of beef on subsequent postmortem aging and shear force. *J. Food Sci.* 55:573.
- FSIS. 1973. *Meat and Poultry Inspection Requirements*. USDA, Washington, DC.
- Koohmaraie, M. 1990a. Inhibition of postmortem tenderization in ovine carcasses through infusion of zinc. *J. Anim. Sci.* 68:1478.
- Koohmaraie, M. 1990b. Quantification of  $\text{Ca}^{2+}$ -dependent protease activities by hydrophobic and ion-exchange chromatography. *J. Anim. Sci.* 68:859.
- Koohmaraie, M. 1992. Effect of pH, temperature, and inhibitors on autolysis and catalytic activity of bovine skeletal muscle  $\mu$ -calpain. *J. Anim. Sci.* 70:3071.
- Koohmaraie, M., A. S. Babiker, A. L. Schroeder, R. A. Merkel, and T. R. Dutson. 1988. Acceleration of postmortem tenderization in ovine carcasses through activation of  $\text{Ca}^{2+}$ -dependent proteases. *J. Food Sci.* 53:1638.
- Koohmaraie, M., J. D. Crouse, and H. J. Mersmann. 1989. Acceleration of postmortem tenderization in ovine carcasses through infusion of calcium chloride: Effect of concentration and ionic strength. *J. Anim. Sci.* 67:934.
- Koohmaraie, M., J. E. Schollmeyer, and T. R. Dutson. 1986. Effect of low-calcium-requiring calcium activated factor on myofibrils under varying pH and temperature conditions. *J. Food Sci.* 51:28.
- Koohmaraie, M., S. C. Seideman, J. E. Schollmeyer, T. R. Dutson, and J. D. Crouse. 1987. Effect of post-mortem storage on  $\text{Ca}^{++}$ -dependent proteases, their inhibitor and myofibril fragmentation. *Meat Sci.* 19:187.
- Koohmaraie, M., and S. D. Shackelford. 1991. Effect of calcium chloride infusion on the tenderness of lambs fed a  $\beta$ -adrenergic agonist. *J. Anim. Sci.* 69:2463.
- Koohmaraie, M., G. Whipple, and J. D. Crouse. 1990. Acceleration of postmortem tenderization in lamb and Brahman-

- cross beef carcasses through infusion of calcium chloride. *J. Anim. Sci.* 68:1278.
- Morgan, J. B., R. K. Miller, F. M. Mendez, D. S. Hale, and J. W. Savell. 1991a. Using calcium chloride injection to improve tenderness of beef from mature cows. *J. Anim. Sci.* 69:4469.
- Morgan, J. B., J. W. Savell, D. S. Hale, R. K. Miller, D. B. Griffin, H. R. Cross, and S. D. Shackelford. 1991b. National beef tenderness survey. *J. Anim. Sci.* 69:3274.
- NRC. 1984. Nutrient Requirements of Beef Cattle (6th Ed.). National Academy Press, Washington, DC.
- SAS. 1989. SAS User's Guide: Statistics. SAS Inst. Inc., Cary, NC.
- St. Angelo, A. J., M. Koohmaraie, K. L. Crippen, and J. D. Crouse. 1991. Simultaneous acceleration of postmortem tenderization/inhibition of warmed-over flavor by calcium chloride-antioxidant infusion into lamb carcasses. *J. Food Sci.* 56:359.
- Vidalenc, P., P. Cottin, M. Merdaci, and A. Ducastaing. 1983. Stability of two  $\text{Ca}^{2+}$ -dependent neutral proteinases and their specific inhibitor during postmortem storage of rabbit skeletal muscle. *J. Sci. Food Agric.* 34:1241.
- Wheeler, T. L., M. Koohmaraie, and J. D. Crouse. 1991. Effects of calcium chloride injection and hot boning on the tenderness of round muscles. *J. Anim. Sci.* 69:4871.
- Whipple, G., and M. Koohmaraie. 1992a. Calcium chloride marination effects on beef steak tenderness and calpain proteolytic activity. *Meat Sci.* (In press).
- Whipple, G., and M. Koohmaraie. 1992b. Freezing and calcium chloride marination effects on beef tenderness and calpastatin activity. *J. Anim. Sci.* 70:3081.
- Whipple, G., M. Koohmaraie, M. E. Dikeman, J. D. Crouse, M. C. Hunt, and R. D. Klemm. 1990. Evaluation of attributes that affect longissimus muscle tenderness in *Bos taurus* and *Bos indicus* cattle. *J. Anim. Sci.* 68:2716.