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ABSTRACT: Our objectives were to examine the effects of prerigor excision and rapid chilling vs. conventional carcass chilling of two muscles on proteolysis and tenderness during the postmortem storage, as well as the effects of fast and slow rates of cooking on myofibrillar characteristics and tenderness. The longissimus thoracis (LT) and triceps brachii (TB), long head muscles were removed 45 min after exsanguination from the left side of 12 carcasses and chilled in an ice bath to induce cold shortening (excised, rapidly chilled). At 24 h postmortem, the corresponding muscles were removed from the right side (conventionally chilled). All muscles were cut into 2.54-cm-thick steaks and assigned to one of two postmortem times (1 or 14 d), and to raw and cooking treatments. Steaks were cooked at 260°C (FAST) or 93°C (SLOW) in a forced-air convection oven to an internal temperature of 70°C. Cooking loss, cooking time, and Warner-Bratzler shear force (WBSF) were measured on cooked steaks. Sarcomere length (SL) and the extent of proteolysis of desmin were measured on raw and cooked steaks. As expected, the excised, rapidly chilled muscles had a much more rapid ($P < 0.05$) temperature decline than those that were conventionally chilled. The excised, rapidly chilled treatment resulted in shorter ($P < 0.05$) SL, and SL was shorter ($P < 0.05$) in LT than in TB steaks. Raw steaks had longer ($P < 0.05$) SL than cooked steaks, regardless of chilling treatment. The FAST cooking resulted in shorter ($P < 0.05$) SL than SLOW cooking in conventionally chilled steaks, but cooking rate had no effect ($P > 0.05$) on SL of rapidly chilled steaks. Generally, TB steaks required longer ($P < 0.05$) cooking times and had higher ($P < 0.05$) cooking losses than LT steaks, and FAST-cooked steaks had greater ($P < 0.05$) cooking losses than SLOW-cooked steaks. Rapidly chilled steaks had less ($P < 0.05$) degradation of desmin than conventionally chilled steaks (31 vs. 41%). Aging for 14 d increased ($P < 0.05$) desmin degradation. Rapid chilling of muscles resulted in much higher ($P < 0.05$) WBSF values, whereas aging resulted in lower ($P < 0.05$) WBSF values. The SLOW-cooked TB steaks were more tender ($P < 0.05$) than FAST-cooked TB steaks and LT steaks cooked at either rate. Excised, rapidly chilled muscles underwent proteolysis, but it occurred at a slower rate during the first 24 h postmortem than it did in conventionally chilled muscles. Cooking rate did not affect tenderness of LT steaks, but SLOW cooking resulted in more tender TB steaks.

Key Words: Beef, Chilling, Cooking, Proteolysis, Tenderness, Rate

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Introduction

Several factors affecting myofibrillar tenderness of meat have been identified and extensively researched. However, the relationships among these factors remain unclear. Sarcomere length and postmortem proteolysis have both been implicated in determining the myofibrillar tenderness of meat (Wheeler and Koohmaraie, 1994; Koohmaraie et al., 1996). However, some studies have reported a weak relationship of sarcomere length with proteolysis and tenderness under some conditions (Gothard et al., 1966; Seideman et al., 1987).

Differences in tenderness exist among muscles differing in location and function in the live animal. These differences are generally attributed to differences in collagen content and/or differences in contraction or stretching during rigor mortis.

In research, the effects of cooking on tenderness are generally considered to be constant; however, studies have shown that cooking methods differing in the rate and medium of heat transfer affect muscle tenderness differently (Lawrence et al., 2001). This suggests that the cooking process can create at least some variation in meat tenderness.

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This study was conducted to evaluate the relationships among factors implicated in determining myofibrillar tenderness and how different muscles and cooking rates affect these relationships. Muscles and treatments used were intended to create variation in tenderness, sarcomere length, connective tissue content, and heating rate to study the interrelationships among these factors and proteolysis in determining beef myofibrillar tenderness. Specifically, our objectives were to quantify the effects of prerigor excision and rapid chilling vs. conventional chilling of two muscles on proteolysis during postmortem storage and the potential impact on tenderness, and to examine the effects of very fast and slow rates of heating on the myofibrillar characteristics and tenderness of muscles that had been either excised and rapidly chilled or conventionally chilled.

Materials and Methods

Muscle Treatments

Charolais × Angus crossbred heifers (n = 12) that had been fed a conventional finishing diet (77.3% corn, 2.5% soybean meal, 2.5% cottonseed hulls, 2.5% molasses, 3.7% vitamin/mineral supplement, and 11.5% corn silage) were selected from a local feeder. Two replications of six heifers were harvested 2 d apart. On the day they were selected, the heifers were transported approximately 90 km to the Kansas State University Meat Laboratory. The heifers were held without feed, but with access to water, overnight and were humanely harvested by conventional means according to Kansas State University's Institutional Animal Care and Use Committee guidelines.

Approximately 45 min postmortem, the longissimus thoracis **(LT)** and the triceps brachii, long head **(TB)** were removed from the left side of each carcass. External fat was removed, and then the muscles were placed in plastic bags and immersed in an ice bath to induce cold shortening (excised, rapidly chilled). The right side was chilled intact in a 0°C cooler equipped with a spray chill (conventional chill). The spray chill cycle emitted water for 1 min, air for 1 min, and was off for 15 min. This cycle was repeated for a total of 8 h. Air chilling was then used for the remainder of the chilling time.

At 24 h postmortem, the right side was ribbed between the12th and 13th ribs. Trained University personnel determined yield and quality grade factors. Following grade data collection, the LT and TB were removed from the right side of each carcass. All muscles were cut into 2.54-cm steaks. Animal and location identity was maintained on all steaks, which were individually vacuum packaged and assigned randomly to a postmortem time (1 or 14 d) and to raw and cooking treatments. Steaks assigned to the 1-d treatment were frozen immediately at –40°C. Steaks assigned to the 14-d aging treatment were aged at 4°C for 14 d, and then frozen at –40°C until further analysis.

pH and Temperature Decline

The decline in muscle temperature and pH was monitored at 1, 2, 4, 8, 12, 16, 20, and 24 h postmortem using an Accumet AP 61 pH meter/thermometer (Fisher Scientific, Pittsburgh, PA). The pH decline of the first replication of six carcasses was monitored with a gel combination spear-type probe (catalog No. 476476, Corning Inc., Corning, NY). Due to equipment failure, pH readings were not taken at 16 and 20 h for the first six carcasses, and the 24-h pH reading was taken on those animals with an IQ 240 pH/mV/thermometer equipped with a nonglass ISEFT sensor probe (IQ Scientific, San Diego, CA). The pH decline on the second replication of six animals was measured using a flat-surface probe (Accumet Flat Surface Polymer Body Electrode, catalog No. 13-620-289, Fisher Scientific) attached to the Accumet AP 61 pH meter. Even though the pH component of the Accumet AP 61 pH meter stopped functioning for carcasses in the first replication, it was used for recording temperature throughout the study.

Cooking Treatments

Cooking was accomplished with a gas, forced-air convection oven (Blodgett, model DFG-102 CH#, G. S. Blodgett Co., Burlington, VT). To achieve a rapid cooking rate, the oven temperature was set at 260°C **(FAST),** whereas to achieve a slow cooking rate, the oven temperature was set at 93°C **(SLOW).** Steaks were thawed for 24 to 36 h prior to cooking, and then placed directly on the oven rack during cooking. Due to even heat and air circulation, uniform cooking was achieved without turning steaks. Internal temperature was monitored with copper constantan thermocouples inserted into the geometric center of the steaks and attached to a Doric model 205 temperature recorder (Vas Engineering, San Francisco, CA). When the internal temperature reached 70°C, the steaks were removed from the oven. Steaks were chilled at 4°C for 24 h before being cored for Warner-Bratzler shear force (**WBSF**) determination. Steaks were weighed before and after cooking to determine cooking loss, and cooking time was recorded.

Warner-Bratzler Shear Force Determination

Six 1.27-cm diameter cores were removed from each cooked, chilled steak parallel to the muscle fiber orientation. Each core was sheared once through the center with an Instron Universal Testing Machine (model 4201, Instron Corp., Canton, MA) equipped with a Warner-Bratzler V-notch blade, a 50-kg compression load cell, and a crosshead speed of 250 mm/min.

Sarcomere Length Measurement

For cooked steaks, a 5-mm portion was removed from the sheared edge of one-half of each of the six cores used for WBSF. For raw steaks, three 1.5 × 1.5 × 2.0-cm

samples (one each from the lateral, center, and medial portions) were removed parallel to the muscle fiber orientation from each steak. Sarcomere length was measured using the protocol of Koolmees et al. (1986). Briefly, samples were fixed in a 5% solution of glutaraldehyde in 0.1 *M* $NaHPO_4$ buffer at pH 7.2 and 4°C. After 4 h, the glutaraldehyde solution was replaced with a 0.2 *M* sucrose solution in 0.1 *M* $NaHPO_4$ buffer at pH 7.2. Samples were held overnight at 4°C. Individual fibers (n = 6 for cooked samples; n = 10 for raw samples) were teased from each sample, placed on a glass microscope slide, and immersed in a drop of sucrose solution. Sarcomere length was measured by passing the beam of a He-Ne laser (model 102-3, Spectra-Physics Inc., Eugene, OR; $\gamma = 0.6328$) through the fiber. The sarcomere length was calculated from the distance between the first order diffraction bands, according to Cross et al. (1981).

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis and Western Blotting Analysis

Samples of raw steaks consisted of approximately 15 g of small muscle pieces, whereas for cooked steaks, SDS-PAGE samples consisted of the remainder of the cores used for WBSF with the outside, cooked edges removed. Muscle protein extraction, SDS-PAGE, Western blotting, and desmin detection were carried out as described by Wheeler and Koohmaraie (1999) and Wheeler et al. (2002). Muscle-specific standards were collected within 30 min of exsanguination from six animals and pooled. All samples from the same muscle and animal were run on the same gel. The appropriate standards were run in the center and outside lanes of each gel. The percentage of desmin remaining in each sample was determined by comparing the intensity of the band produced by that sample with the mean intensity of the bands produced by the three standards run on the same gel. Detection of protein was accomplished using a Pierce SuperSignal West Dura Extended Duration Substrate Kit (Pierce Endogen, Rockford, IL). Membranes were exposed for 3 min and then an image was captured using ChemiImager 4000 digital imaging system (Alpha Innotech, San Leandro, CA). The extent of desmin degradation was expressed as the difference in the density of the protein band from each sample relative to the at-death reference standard.

Some of the LT samples produced density values that were greater than the at-death standards (i.e., a value for desmin remaining greater than 100 was obtained). When this occurred, the sample with the largest density value was set at 100%. The remaining samples on that gel were adjusted accordingly.

Statistical Analysis

The pH and temperature decline data were analyzed as a split-plot design with repeated measures. The whole plot experimental unit was animal, with muscle as the treatment. The subplot experimental unit was muscle, and the treatment was chilling. These data were analyzed with the Proc MIXED procedure of SAS (SAS Inst., Inc., Cary, NC). The fixed effects included muscle and chilling treatment. Random effects included animal and chilling treatment within animal. Because two different pH probes were used, pH probe was included as a random effect for the pH data. Because the treatment × time interaction for these two traits was deemed to be important, least squares means for that interaction were generated and separated using the DIFF option with a Scheffe adjustment.

The sarcomere length, WBSF, desmin degradation, and cooking traits data were analyzed as a split-split plot design with a 2 × 2 factorial subplot. The whole plot experimental unit was animal and the treatment was muscle. The subplot experimental unit was muscle and the treatment was chilling. The sub-subplot experimental unit was steak and the treatments were aging and cooking rate. The data were analyzed with the Proc MIXED procedure of SAS. Fixed effects were muscle, chilling treatment, aging treatment, and cooking treatment. Random effects were animal and chilling treatment within animal. Least squares means were generated for significant interactions, and main effects not involved in higher order interactions were separated with the PDIFF option. Additionally, the changes in WBSF and percentage of desmin degradation during aging were calculated by subtracting the 14-d values from 1-d values.

Table 1. Simple means and standard deviations for carcass traits

Trait	Mean	SD
Live weight, kg	512	32
Hot carcass weight, kg[a]	320	15
Dressing percentage	62.0	0.02
Adjusted fat thickness, cm	1.4	0.3
Longissimus muscle area, cm^2	89.5	8.4
Estimated kidney, pelvic, and heart fat, %	1.9	0.4
USDA yield grade	2.5	0.6
Lean maturity[b]	175.0	16.0
Skeletal maturity[b]	163.0	16.2
Overall maturity[b]	168.0	12.5
Marbling score[c]	555.0	82.3

[a]Hot carcass weight was determined by doubling the weight of the right (conventionally chilled) side.
[b]$100 = A^{00}$, $200 = B^{00}$.
[c]$500 = Small^{00}$, $600 = Modest^{00}$.

Results and Discussion

For characterization purposes, the simple statistics for the carcass traits of the animals used in this study are presented in Table 1. These heifers produced carcasses that are typical of Charolais-crossbred cattle that would be found in a commercial slaughter facility.

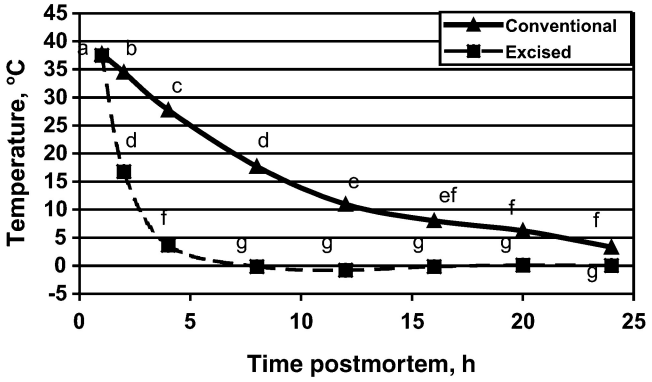

Figure 1. Least squares means for temperature decline of excised, rapidly chilled and conventionally chilled beef longissimus thoracis and triceps brachii, long head muscles. [abcdefg]Means that do not have a common superscript letter differ ($P < 0.05$).

Chilling Rate Effects

Temperatures of rapidly chilled muscles were not different from the conventionally chilled muscles at 1-h postmortem; however, the subsequent temperature decline was much faster in the rapidly chilled muscles (Figure 1). By 2 h postmortem, the rapidly chilled muscles had reached a temperature of 16.8°C, whereas the conventionally chilled muscles did not approach that temperature until 8 h postmortem. The rapidly chilled muscles had a temperature of 3.7°C at 4 h postmortem, whereas the conventionally chilled muscles had a temperature of 3.6°C at 24 h postmortem.

The pH declines presented in Figure 2 show that muscle pH was not statistically different ($P > 0.05$) between the chilling regimens at any specific time postmortem, but the rapidly chilled muscles had numerically higher pH values between 2 and 16 h postmortem.

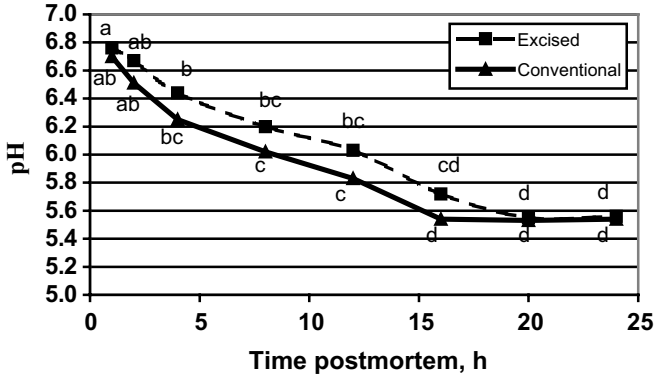

Figure 2. Least squares means for pH decline of excised, rapidly chilled and conventionally chilled beef longissimus thoracis and triceps brachii, long head pooled muscles. [abcd]Means that do not have a common superscript letter differ ($P < 0.05$).

Table 2. Least squares interaction means for sarcomere length of excised rapidly chilled or conventionally chilled beef longissimus thoracis and triceps brachii, long head muscles

Item	Chilling treatment	Sarcomere length, μm
Longissimus thoracis	Conventional	1.78[x]
Longissimus thoracis	Excised, rapid	1.27[z]
Triceps brachii, long head	Conventional	2.07[w]
Triceps brachii, long head	Excised, rapid	1.37[y]
SEM		0.03
$P > F$		0.0001

[w,x,y,z]Means that do not have a common superscript letter differ ($P < 0.05$).

After 20 h, the pH was essentially identical between treatments. The excised, rapidly chilled and the conventionally chilled muscles had ultimate pH values of 5.56 and 5.54, respectively.

Prerigor excision and the differences in temperature and pH decline were adequate to cause cold shortening ($P < 0.05$; Table 2) in the excised, rapid-chilling treatment. Additionally, an interaction between muscle and chilling treatment was observed for sarcomere length. The conventionally chilled TB had longer ($P < 0.05$) sarcomeres than did the conventionally chilled LT; conversely, the TB cold shortened to a greater extent ($P < 0.05$) than the LT (34 vs. 29%, respectively). Drastic shortening associated with rapid chilling of unrestrained muscle is consistent with the findings of Locker and Hagyard (1963), Marsh and Leet (1966), and Honikel et al. (1983). Geesink et al. (1995) reported a larger difference between the sarcomere lengths of these two muscles than we found with conventional chilling. Based on sarcomere length data, it is clear that the objective of creating a severely contracted state with which to study the effects of sarcomere length on tenderness and tenderization was met.

Drastic toughening caused by cold shortening has been reported in numerous studies (Locker, 1960; Marsh et al., 1968; Bouton et al., 1973). The shortening induced by our excised, rapidly chilled treatment approached the 40% shortening at which maximal toughening was observed by Marsh and Leet (1966) and Davey and Gilbert (1977). It is commonly believed that the toughening associated with shortening is a manifestation of greater protein density caused by greater overlapping of the thick and thin filaments. It is thought that 35 to 40% shortening corresponds to the maximal overlap of the thick and thin filaments (Marsh and Carse, 1974). As expected, our excised, rapid-chilling treatment drastically increased ($P < 0.01$) WBSF values in our study, regardless of aging or cooking treatment (9.9 vs. 4.8 kg; Table 3). Based on the magnitude of shortening, it is surmised that the toughening observed in our study approached the maximum and was similar to that reported for the longissimus by Wheeler and Koohmaraie (1999).

Table 3. Least squares main-effect means for desmin degradation, Warner-Bratzler shear force (WBSF), and cooking loss for excised, rapidly chilled and conventionally chilled longissimus thoracis and triceps brachii, long head muscles

Treatment	Desmin degraded, %[a]	WBSF, kg	Cooking loss, %
Chilling treatment effect			
Conventional	40. 9[z]	4.8[y]	26.0
Excised, rapid	30.5[y]	9.9[z]	25.2
SEM	2.61	0.28	0.55
$P > F$	0.0008	<0.0001	0.23
Aging effect			
1 d	—	7.8[z]	24.9[y]
14 d	—	6.8[y]	26.2[z]
SEM	—	0.25	0.55
$P > F$	—	<0.001	0.06

[a]Determined by comparing samples to an at-death standard.
[y,z]Means within a column and main effect that do not have a common superscript letter differ ($P < 0.05$).

The proteolysis of desmin was reduced ($P < 0.05$) in excised, rapidly chilled muscle, regardless of aging or cooking treatment (Table 3). This is in contrast to Koohmaraie et al. (1984) and Wheeler and Koohmaraie (1999), who reported no difference in proteolysis between cold-shortened and normal muscle.

The interaction means (Table 4) show a small increase ($P < 0.05$) in the sarcomere length of the TB during aging. However, no increase ($P > 0.05$) in sarcomere length of the LT was found during aging. Increased sarcomere length during aging has been reported by Stromer and Goll (1967a,b) and Wheeler and Koohmaraie (1994). Wheeler and Koohmaraie (1999) and Geesink et al. (2001) found no increase in sarcomere length with aging, and Geesink et al. (2001) suggested that increased sarcomere lengths reported with aging in some studies could be due to sampling error.

The proteolytic degradation of cytoskeletal proteins, including desmin, is commonly reported to increase with postmortem storage (Koohmaraie, 1994; Huff-Lonergan et al., 1996). In our study, the extent of the proteolysis of desmin increased ($P < 0.01$) with aging for both muscles (Table 4). However, at 1 d postmortem, the TB tended ($P = 0.06$) to have a greater proportion of desmin degraded, but after 14 d, the LT tended ($P = 0.12$) to have more of its desmin degraded, thus the statistically significant ($P < 0.05$) interaction. Additionally, the mean difference in the extent of proteolysis between d 1 and d 14 was greater ($P < 0.05$) in LT steaks than in TB steaks (Table 4).

Table 4. Least squares interaction means pooled across chilling treatments for sarcomere length and percentage of desmin degraded at different times postmortem in longissimus thoracis and triceps brachii, long head muscles

Muscle	Aging time, d	Sarcomere length, μm	Desmin degraded, %[a]
Longissimus thoracis	1	1.53[z]	19.3[y]
Longissimus thoracis	14	1.51[z]	51.6[x]
Triceps brachii, long head	1	1.70[y]	25.9[y]
Triceps brachii, long head	14	1.74[x]	46.1[x]
SEM		0.02	2.97
$P > F$		0.02	0.0031

[a]Determined by comparing samples to an at-death standard.
[x,y,z]Means that do not have a common superscript letter differ ($P < 0.05$).

Even though proteolysis was reduced in the excised, rapidly chilled muscles, it is important to note that the mean change in the percentage of desmin that had been degraded between 1 and 14 d was not different ($P > 0.05$) between chilling treatments (Table 5). This indicates that the difference in proteolysis between treatments occurred during the first 24 h, after which, the rate of proteolysis did not differ.

A possible explanation for the reduced proteolysis observed during the first 24 h in our study is the much faster chilling for the excised muscles (Figure 1). The rapidly chilled muscles also declined to a lower ultimate temperature than the control samples. Decreasing temperature has been shown to lower the proteolytic activity of μ-calpain (Koohmaraie et al., 1986) and to slow the rate of tenderization in muscle (Davey and Gilbert, 1976; Whipple et al., 1990; Simmons et al., 1996). Wheeler and Koohmaraie (1999), who found no difference in proteolysis between normal and shortened muscle, induced shortening in a manner that did not affect the temperature decline of the muscles studied. Those findings, in conjunction with our results, support the theory that the reduction in proteolysis was due to reduced temperature rather than sarcomere shortening itself.

Aging improved ($P < 0.01$) WBSF values of both muscles, regardless of shortening treatment (Table 5). The improvement in tenderness with postmortem storage is common (Bouton et al., 1975; Locker and Wild, 1982; Campo et al., 2000). However, reports on the effect of aging on cold-shortened meat are varied. Goll et al. (1964), Herring et al. (1967), and Bouton et al. (1973) found that aging cold-shortened beef improved tenderness, whereas other studies found no improvement in tenderness as a result of aging (Davey et al., 1967; 1976; Locker, 1982).

Locker (1982) suggested that the gap filaments (titin and nebulin) were the primary determinants of meat tenderness. Other authors have also presented evidence that these two proteins are important to tenderness (Taylor et al., 1995; Huff-Lonergan et al., 1996; Koohmaraie, 1996). According to Locker (1982), cold-shortened meat does not improve in tenderness because the overlapping of the thick and thin filaments does not allow the calpain enzymes access to these proteins. If this were true, postmortem tenderization would not occur in cold-shortened muscle. However, improvements in the tenderness of cold-shortened meat with aging have been reported. Perhaps this disagreement

Table 5. Least squares main effect and interaction means for the change in desmin degradation and Warner-Bratzler shear force (WBSF) in excised, rapidly chilled and conventionally chilled longissimus thoracis and triceps brachii, long head steaks between 1 and 14 d of aging

Treatment	Chilling treatment	Change in desmin degraded, %[a]	Change in WBSF, kg
Chilling treatment main effect			
	Conventional	24.93	0.71
	Excised, rapid	28.08	1.38
	SEM	3.92	0.29
	$P > F$	0.56	0.12
Muscle main effect			
Longissimus thoracis		32.76	1.01
Triceps brachii, long head		20.26	1.09
SEM		3.82	0.28
$P > F$		0.02	0.82
Muscle × chilling treatment interaction			
Muscle			
Longissimus thoracis	Conventional	33.59	1.07[yz]
Longissimus thoracis	Excised, rapid	31.92	0.94[yz]
Triceps brachii, long head	Conventional	16.28	0.37[z]
Triceps brachii, long head	Excised, rapid	24.24	1.82[y]
SEM		5.26	0.40
$P > F$		0.34	0.05

[a]Determined by comparing samples to an at-death standard.
[y,z]Within a main effect or interaction, means that do not have a common superscript letter differ ($P < 0.05$).

can be partially explained by the findings of Davey and Gilbert (1977) and Locker and Wild (1982), who found that shear force and yield point (the point at which integrity is lost with longitudinal stretching) were highly correlated in normal muscle, and both shear force and yield point decreased with postmortem storage. In cold-shortened muscle, however, yield point decreased with aging but shear force did not (Locker and Wild, 1984). It has been suggested that yield point is an indicator of weakening of the cytoskeletal proteins in the I-band (Locker and Wild, 1982); thus, yield point would be an indicator of the proteolysis of titin and nebulin. It is suggested, then, that the reason some workers have not observed tenderization with aging in cold-shortened muscle is that the tenderization in response to proteolysis is masked by the overlapping thick and thin filaments.

In our study, postmortem storage improved WBSF of muscles, regardless of chilling treatment (Table 5). The change in WBSF during aging was greatest ($P < 0.05$) in the excised, rapidly chilled TB steaks and least in the conventionally chilled TB steaks. No difference ($P > 0.05$) in the rate of change in WBSF during aging was observed between conventionally chilled and excised, rapidly chilled LT steaks. Even though tenderization occurred to a greater extent in the excised, rapidly

Table 6. Least squares interaction means for cooking time of excised, rapidly chilled and conventionally chilled longissimus thoracis or triceps brachii (long head) steaks aged for 1 or 14 d and cooked at two rates

Treatment	Chilling treatment	Aging time, d	Cooking time, min	
			FAST[a]	SLOW[b]
Longissimus thoracis	Conventional	1	13.4[z]	70.5[y]
Longissimus thoracis	Conventional	14	16.4[z]	70.1[y]
Longissimus thoracis	Excised, rapid	1	15.1[z]	68.4[y]
Longissimus thoracis	Excised, rapid	14	16.6[z]	85.5[x]
Triceps brachii, long head	Conventional	1	19.4[z]	97.4[w]
Triceps brachii, long head	Conventional	14	20.2[z]	104.3[w]
Triceps brachii, long head	Excised, rapid	1	19.7[z]	98.3[w]
Triceps brachii, long head	Excised, rapid	14	19.7[z]	84.1[x]
Pooled SEM			4.00	4.00

[a]Cooked in a forced air convection oven at 260°C.
[b]Cooked in a forced air convection oven at 93°C.
[w,x,y,z]Means that do not have a common superscript letter differ ($P < 0.05$).

Table 7. Least squares interaction means for sarcomere length of excised rapidly chilled and conventionally chilled longissimus thoracis and triceps brachii, long head steaks cooked at two rates

Chilling treatment	Cooking treatment	Sarcomere length, μm
Conventional	Raw	2.02[v]
Conventional	FAST[a]	1.84[x]
Conventional	SLOW[b]	1.91[w]
Excised, rapid	Raw	1.35[y]
Excised, rapid	FAST[a]	1.31[z]
Excised, rapid	SLOW[b]	1.29[z]
SEM		0.02
$P > F$		<0.0001

[a]Cooked in a forced-air convection oven at 260°C.
[b]Cooked in a forced-air convection oven at 93°C.
[v,w,x,y,z]Means that do not have a common superscript letter differ ($P < 0.05$).

chilled vs. conventionally chilled TB muscle, the toughening effect of cold shortening was not eliminated by aging (Table 5). Geesink et al. (1995) reported small improvements in the tenderness of the TB compared with that of the longissimus. Perhaps the longer sacromere length in the conventionally chilled TB muscles reduces the impact of proteolysis on tenderness. When shorter sarcomeres are present, the impact of proteolysis is more pronounced.

Cooking Rate Effects

As expected, SLOW cooking required much longer ($P < 0.05$) cooking times than FAST cooking (Table 6). There was no difference ($P > 0.05$) in cooking time due to muscle, chilling treatment, or aging treatment when steaks were cooked FAST. However, when SLOW cooking was used, the TB steaks generally required longer ($P < 0.05$) cooking times than the LT steaks.

A chilling treatment × cooking treatment interaction was observed for sarcomere length (Table 7). Cooking at both temperatures caused a reduction ($P < 0.05$) in sarcomere length. In conventionally chilled muscle, raw steaks had the longest sarcomeres. FAST cooking caused the greatest reduction in sarcomere length, with sarcomeres for SLOW cooking being intermediate to those of the raw and FAST cooked steaks. Reduced sarcomere length associated with cooking has been consistently reported (Lewis et al., 1977; Cheng and Parrish, 1979). Cooking also shortened the sarcomeres of the excised, rapidly chilled muscles but no difference ($P > 0.05$) was found between the two cooking treatments for conventionally chilled muscles (Table 7). This is in agreement with Hegarty and Allen (1975), Locker and Daines (1975), and Wheeler and Koohmaraie (1999), who reported that muscle shortening that resulted from cooking was dependent upon the sarcomere length in the raw state.

A muscle × cooking treatment interaction was found for the degradation of desmin, WBSF, and cooking loss (Table 8). The raw LT steaks and SLOW-cooked TB steaks underwent more degradation of desmin than FAST-cooked LT steaks. Koohmaraie et al. (1986) found that the activity of μ-calpain at 5°C (pH 7.5) was about 60% of the maximal activity (25°C, pH 7.5). At a pH of 5.5, μ-calpain activity was lower at both temperatures and the difference in activity due to temperature was smaller. However, Inomata et al. (1984) reported that μ-calpain retained as much as 85% of its activity with heating up to 60°C (pH 7.5). Consistent with those results, Wheeler et al. (2002) reported greater desmin degradation in d-14 beef after belt-grill cooking than in raw samples, but did not detect that effect in pork, likely because 78% of desmin was already degraded in the raw pork samples. Furthermore, Davey and Gilbert (1976) found that the rate of tenderization of meat was maximized at 60°C, but approached zero at 70°C. Therefore, we hypothesized that extremely slow heating rates would increase the time that the meat was at elevated temperatures capable of producing rapid proteolysis. The SLOW-cooked LT and TB steaks combined had numerically greater amounts of desmin degradation (Table 8), but these differences were not statistically significant. The SLOW cooked TB steaks did have greater ($P < 0.05$) proteolysis of desmin than the FAST-cooked LT. We cannot explain the observation that, at 1 d postmortem, the RAW LT steaks had greater desmin degradation than the FAST-cooked LT steaks (Table 8). This could have been due to sampling variation.

No difference ($P > 0.05$) was observed between cooking treatments for WBSF for LT steaks (Table 8). Furthermore, LT steaks were not different ($P > 0.05$) from TB steaks when cooked FAST. However, SLOW cooking caused a reduction ($P < 0.05$) in WBSF for TB steaks. The small difference in proteolysis between cooking methods may have contributed to the increase in tenderness of the TB due to cooking rate. Greater sarcomere shortening due to FAST cooking was observed in both muscles as discussed earlier. Because no difference in tenderness was found between cooking rates in LT steaks, it is not likely that changes in sarcomere length resulting from cooking caused the differences in tenderness of the TB.

Slower cooking rates have been shown to improve tenderness (Bayne et al., 1969; Cross et al., 1976; Lawrence et al., 2001). The myofibrillar contribution to shear force was influenced by the heating rate up to 80°C (Møller, 1981). However, the tenderizing effect of slow cooking is commonly attributed to the solubilization of collagen (Bayne et al., 1969; Penfield and Meyer, 1975). Møller (1981) found the connective tissue contribution to shear force was primarily influenced by a heating rate between 60°C and 80°C. Hence, the greater collagen content of the TB muscles (McKeith et al., 1985) in our study could be responsible for the differing response to cooking rate. The time at which steaks were in this temperature range was extended by our SLOW cooking treatment.

Table 8. Least squares interaction means for desmin degradation, Warner-Bratzler shear force (WBSF), and cooking losses of longissimus thoracis and triceps brachii, long head steaks cooked at two rates

Muscle	Cooking treatment	Desmin degraded, %[a]	WBSF, kg	Cooking loss, %
Longissimus thoracis	Raw	40.10[x]	—	—
Longissimus thoracis	FAST[c]	31.76[y]	7.54[x]	27.10[y]
Longissimus thoracis	SLOW[d]	34.32[xy]	7.55[x]	19.83[z]
Triceps brachii	Raw	33.20[xy]	—	—
Triceps brachii	FAST[c]	35.54[xy]	7.57[x]	29.71[x]
Triceps brachii	SLOW[d]	40.26[x]	6.62[y]	25.65[y]
	SEM	3.29	0.32	0.74
	$P > F$	0.03	0.03	0.02

[a]Determined by comparing samples to an at death standard.
[c]Cooked in a forced air convection oven at 260°C.
[d]Cooked in a forced air convection oven at 93°C.
[x,y,z]Means within a column that do not have a common superscript letter differ ($P < 0.05$).

FAST cooking caused greater ($P < 0.05$) cooking losses than SLOW cooking in both muscles (Table 8). This is consistent with the results of Cross et al. (1976), who reported that faster heating rates caused greater evaporative and total cooking losses. Lawrence et al. (2001) found that cooking steaks from five beef muscles on an electric-belt grill at 163°C resulted in higher ($P < 0.05$) cooking losses than cooking on the belt grill at 93°C, even though cooking was relatively rapid at either temperature because of the efficient heat transfer of the belt grill.

The difference in cooking losses between cooking rates was much greater ($P < 0.05$) in LT steaks compared to TB steaks (7.27 and 4.06 percentage points for FAST and SLOW, respectively). However, the TB steaks had higher cooking losses than the LT steaks when cooked at the same rate. This could be due to the higher connective tissue content of the TB (McKeith et al., 1985). Davey and Gilbert (1974) found that the temperature at which cooking loss increased in meat corresponded to the temperature at which isolated collagen shrunk. It seems reasonable that the differences in cooking losses between cooking rates observed in our study could be due to a difference in the force generated on the myofibrils by collagen shrinkage. The collagen shrinkage might not have been as severe and, therefore, unable to generate as much force before being solubilized in the SLOW-cooked steaks.

Implications

Cold shortening caused by excision and rapid chilling drastically increases the toughness of meat. Postmortem proteolysis will proceed in cold-shortened muscle and result in tenderization. However, the conditions that cause cold shortening likely will decrease the rate and extent of proteolysis during the first 24 h postmortem, and meat will not tenderize sufficiently to become equal to normal muscle. Extremely rapid heating in a convection oven may be detrimental to the tenderness of muscles high in connective tissue and may cause increased cooking loss, which would result in a less juicy product. Slow rates of heating may improve the tenderness of excised, rapidly chilled muscles that are moderate to high in connective tissue.

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