

EFFECTS OF HIGH-TEMPERATURE CONDITIONING ON ENZYMATIC ACTIVITY AND TENDERNESS OF *BOS INDICUS* LONGISSIMUS MUSCLE^{1,2}

G. Whipple³, M. Koohmaraie⁴, M. E. Dikeman^{3,5}
and J. D. Crouse⁴

Kansas State University³, Manhattan 66506 and
U.S. Department of Agriculture⁴, Clay Center, NE 68933

ABSTRACT

We studied the effects of high-temperature conditioning (HTC) on beef longissimus (LM) and semitendinosus muscles. Eleven 5/8 Sahiwal × Angus, Hereford or Angus × Hereford crosses (seven heifers and four steers) were slaughtered. Alternate carcass sides were held at 22 ± 3°C for 6 h, then chilled at -1°C for 18 h. The opposite, control (C) sides were chilled at -1°C for 24 h. Samples were removed only from the LM at various times to determine calcium-dependent protease (CDP) and CDP inhibitor (INH) activity, cathepsins B and B + L activity, shear-force, sensory panel traits, myofibrillar fragmentation index (MFI) and sarcomere length. Results were analyzed by least squares procedures; our model included fixed effects of temperature, sex and their interaction. The LM temperature remained higher ($P < .01$) for the HTC treatment at 3, 6, 9 and 12 h postmortem. In addition, HTC increased the rate of pH decline, which resulted in pH differences ($P < .01$) at 6, 9 and 12 h. At d 1, LM steaks had lower ($P < .05$) shear forces (8.3 vs 9.6 kg) from HTC than C carcasses. At d 14, LM shear forces tended ($P = .13$) to be lower for HTC (6.9 kg) than for C (7.7 kg) carcasses. At 3, 7 and 14 d, MFI for LM were greater ($P < .07$) for the HTC steaks. However, by 6 h postmortem, INH activity had decreased ($P < .10$) 35% in HTC samples, but no change had occurred in C samples ($P > .10$). At 24 h postmortem, INH activity was 55 and 40% lower ($P < .01$) than the initial activity in HTC and C sides, respectively. By 6 and 24 h postmortem, HTC had an 81 and 88% loss in CDP-I activity, whereas activity in C declined 62 and 65%, respectively. Thus, HTC propagated a more rapid decline ($P < .01$) in CDP-I activity, with only a slight effect on CDP-II activity. These results suggest that HTC marginally improved LM tenderness of 5/8 Sahiwal crosses. The HTC effect on CDP-I and INH activities may be related to the tenderness improvement of d 1, possibly by providing a more suitable environment for proteolysis by CDP-1.

(Key Words: Beef, High-Temperature Conditioning, Tenderness, Proteases, Aging.)

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Introduction

Many studies have evaluated changes that occur in muscle during postmortem storage and their relationship with meat tenderization. Higher carcass temperatures early in the postmortem period speed the aging process and result ultimately in increased tenderness (Lochner et al., 1980; Marsh et al., 1981). Several mechanisms may explain this effect. A more rapid decrease in pH at higher tempera-

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³Dept. of Anim. Sci. and Ind.

⁴Roman L. Hruska U.S. Meat Anim. Res. Center, ARS, USDA, Clay Center, NE.

⁵To whom reprint requests should be addressed.

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tures may rupture the lysosomal membrane (Moeller et al., 1976) in which some cathepsins could hydrolyze specific myofibrillar proteins. Also, the combination of low pH and high temperature could promote an earlier release of Ca^{2+} from the sarcoplasmic reticulum, thus activating calcium-dependent protease-I. Although calcium-dependent protease-I retains only 24 to 28% of its maximum activity at postmortem conditions of pH 5.5 to 5.8 and 5°C (Koochmaraie et al., 1986), calcium-dependent protease-I hydrolyzes those myofibrillar proteins that change with postmortem storage (Goll et al., 1983; Koochmaraie, 1988). Some researchers have studied the effects of temperature on in vitro enzyme activity, whereas others have studied its relationship with muscle tenderness in whole carcasses and with myofiber integrity. However, most studies have been conducted with animals not possessing a tenderness problem. Because meat from *Bos indicus* breed crosses often is less tender than meat from *Bos taurus* breeds (Koch et al., 1976; McKeith et al., 1985; Crouse et al., 1987, 1989), we studied whether tenderness could be altered by carcass high-temperature conditioning and, if so, what mechanisms are involved.

Materials and Methods

Experimental Animals. Seven heifers and four steers of 5/8 Sahiwal \times Angus, Hereford or Angus \times Hereford weighing an average of 448 kg were slaughtered at 15 to 17 mo of age. Carcasses were not electrically stimulated. Within 1 h postmortem (0 h), longissimus muscle (LM) samples were removed from alternate left and right sides ($n = 11$) from each carcass at the fifth to sixth rib region. Seven of the sides sampled at 0 h and four of those not sampled at 0 h were high-temperature conditioned (HTC) at $22^\circ\text{C} \pm 3^\circ\text{C}$ for 6 h, then chilled at -1°C for 18 h. The opposite, control (C) sides were chilled at -1°C for 24 h. At 6 h, LM samples were taken at the same location on opposite sides from those in which the previous (0 h) samples had been removed, which allowed 6-h samples to be taken from both the HTC ($n = 4$) and C ($n = 7$) sides. At 24 h postmortem, the LM and semitendinosus (ST) muscles were removed from both carcass sides ($n = 22$). The design was completely randomized because sampling time was not the same for both carcass sides. Longissimus

steaks 2.54 cm thick were cut, vacuum-packaged and aged at 4°C for each of three aging times (3, 7 and 14 d), whereas ST steaks were aged for 14 d only.

Temperature and pH Determinations. Temperature of the LM and ST and pH of the LM were determined < 1 h postmortem and were monitored at 3-h intervals for 12 h. Temperature and pH also were recorded at 24 h. For pH measurements, 2.5 g of tissue were homogenized in 25 ml of a sodium iodoacetate-150 mM KCl solution, pH 7.0 (Bendall, 1973) for 10 s using a Polytron at full speed. Temperature of the LM was monitored using an electronic digital probe at the 11th rib region. Three temperature measurements were recorded at approximately 5.7, 2.5 and .3 cm depths from the dorsal edge of the LM, and an average temperature was calculated. Temperatures of the ST were determined by a thermometer located in the center of the muscle for 24 h.

Calcium-Dependent Protease and Inhibitor Activities. Activities of calcium-dependent protease (CDP)-I and -II and CDP inhibitor (INH) were determined on fresh LM at 0, 6 and 24 h postmortem according to the procedures of Koochmaraie (1990). Activities were expressed as the amount of CDP caseinolytic activity in 100 g of muscle. One unit of CDP-I and -II activity was defined as the amount of enzyme that catalyzed an increase of 1.0 absorbance unit at 278 nm in 1 h at 25°C. One unit of INH activity was defined as the amount that inhibited one unit of DEAE-sephacel-purified CDP-II activity.

Cathepsin B and B + L Activities. Lysosomal-enriched fractions were prepared from 5 g of LM taken at 6 h, 1 and 14 d postmortem, using methods of Bechet et al. (1986). Protein determination was according to procedures of Markwell et al. (1978). Activities of cathepsins B and B + L were assayed (Kirscke et al., 1983) using amino-methyl coumarin as a fluorescent tag on the substrates, Z-Arg-Arg-NMec and Z-Phe-Arg-NMec (where Z = benzyloxycarbonyl and NMec = 4-methyl-7-coumarylamide) with a 30-min incubation at 37°C. Specific activities were expressed as $\text{pmole}\cdot\text{mg}^{-1}\cdot\text{min}^{-1}$.

Myofibril Fragmentation Index Measurements. Samples were excised from the LM at 24 h postmortem and were vacuum-aged for 3, 7 and 14 d. Myofibril fragmentation indices (MFI) were determined on unfrozen muscle at

TABLE 1. SEX AND OVERALL LEAST SQUARES MEANS (\pm SE) FOR CARCASS TRAITS

Traits	Heifer	Steer	Overall
Hot carcass wt, kg	264 \pm 11 ^d	302 \pm 14 ^e	283 \pm 9
Adjusted fat thickness, cm	1.3 \pm .1	1.1 \pm .2	1.2 \pm .1
Ribeye area, cm ²	64.3 \pm 3.1 ^d	76.8 \pm 4.1 ^e	70.5 \pm 2.5
Marbling score ^a	332 \pm 45	384 \pm 59	358 \pm 37
Kidney, pelvic, heart fat, %	2.8 \pm .2	2.4 \pm .2	2.4 \pm .2
Yield grade	3.3 \pm .2	2.8 \pm .3	3.1 \pm .2
Quality grade ^b	190 \pm 17	203 \pm 19	205 \pm 20
Overall maturity ^c	161 \pm 4	155 \pm 4	159 \pm 5

^aUSDA marbling score: 200–299 = slight, 300–399 = small.

^bUSDA quality grade: 100–199 = Select, 200–299 = Choice.

^cUSDA maturity score: 100–199 = A maturity.

^{d,e}Means between heifers and steers without a common superscript letter differ ($P < .05$).

1, 3, 7 and 14 d postmortem by the method of Culler et al. (1978).

Sarcomere Length Measurements. Sarcomere lengths of fixed, previously frozen LM (center of muscle) excised at 24 h postmortem were measured using a neon laser (Cross et al., 1981).

Myofibril Isolation and SDS-PAGE. Myofibrils were isolated according to Olson et al. (1976) at 0 h, 6 h, 1 and 14 d postmortem. Protein concentration was determined using the biuret method. Electrophoretic procedures followed those of Laemmli (1970). Myofibrillar proteins were separated using a discontinuous gradient slab gel with 7.5 to 15% acrylamide. The acrylamide to bisacrylamide weight ratio was 37.5:1.

Warner-Bratzler Shear and Sensory Panel Evaluations. At 24 h postmortem, six steaks, 2.54 cm thick, were removed from the loin beginning at approximately the first lumbar vertebra, vacuum-packaged, aged for 1 or 14 d and frozen at -30°C . Steaks were thawed and cooked on Farberware open-hearth broilers to an internal endpoint temperature of 70°C . For Instron Warner-Bratzler shear (WBS) determinations, steaks were tempered for approximately 24 h at refrigerator temperature. Six 1.3-cm cores were removed parallel to the muscle fibers with a hand coring device. For sensory-panel tenderness scores, cooked steaks were foil-wrapped and held at 70°C (< 30 min) in a preheated oven. In four sessions (two slaughter groups/session), eight panelists evaluated 3 ($1.3 \times 1.3 \times 2.5$ cm) cubes/steak following AMSA (1978) guidelines, in which juiciness, ease of fragmentation, connective tissue amount, overall tenderness and off-flavor were scored.

Statistical Analysis. The analysis of variance, performed using the General Linear Models procedure of SAS (1985), included the fixed effects of temperature, sex and their interaction. Means were separated using least squares procedures.

Results and Discussion

Heifer carcasses were lighter and had smaller ribeyes ($P < .05$) than steer carcasses, but there were no differences for marbling score or fat thickness (Table 1). As expected, temperatures remained higher ($P < .01$) in the HTC ST muscle at 3, 6 and 9 h postmortem and the LM at 3, 6 9 and 12 h than in C muscles (Figure 1). By 24 h, the HTC muscles

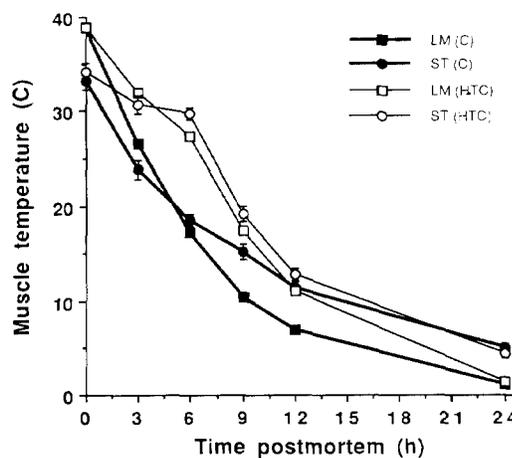


Figure 1. Temperature declines in longissimus and semitendinosus muscles. Bars represent SE of the mean.

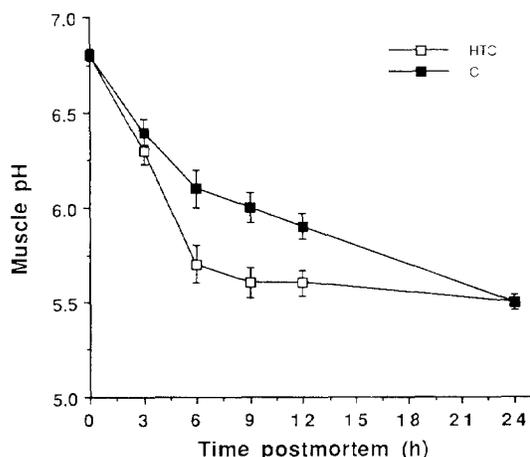


Figure 2. Longissimus pH declines. Bars represent SE of the mean.

had cooled to the temperature of the C muscles. Figure 2 indicates that the LM pH of HTC sides was lower ($P < .01$) than the pH of C sides at 6, 9 and 12 h postmortem. However, ultimate pH was unaffected by HTC, which agrees with Newbold and Harris (1972), who found that temperature postmortem had only a small effect on ultimate pH. Crouse and Seideman (1984) also found 3-h pH to be lower in carcasses held at 26°C than those carcasses held at 1 or 12°C.

No tenderness differences ($P > .10$) were detected at d 1 or at d 14 in ST steaks. On the other hand, LM steaks from HTC sides had lower WBS values ($P < .05$) than C steaks at d 1 postmortem (Table 2). However, sensory-panel scores failed to reveal this difference. Neither WBS values nor sensory panel scores were different ($P > .10$) for LM steaks at d 14. Our study revealed a greater ($P < .05$) off-flavor intensity in the HTC steaks at d 14 (Table 2). It appears that the higher temperature treatment increased the rate of tenderization in the LM but not in the ST. However, the LM tenderness improvement still was not sufficient for any of the steaks to have a WBS value ≤ 5.0 kg. Generally, a WBS value ≥ 5.0 kg is considered unacceptable in tenderness. Dransfield et al. (1981) observed that a 5°C rise in temperature increased the rate of tenderization in several beef muscles, including the LM. Also, Yu and Lee (1986) reported that delayed chilling at 25°C for 8 h increased LM tenderness. In grass-fed cattle, Lee and

Ashmore (1985) found that LM steaks from HTC sides were more tender at d 3 but not at d 7. However, Smith et al. (1979) noted that chilling carcasses of grass-fed cattle at 13°C rather than at 3°C did not accelerate postmortem tenderization. In addition, Crouse and Seideman (1984) found no differences in tenderness in grain- or grass-fed H \times A heifer carcasses chilled at either 1, 12 or 26°C.

Tenderness often is related to the amount of muscle fiber fragmentation, as indicated by MFI values (Olson and Parrish, 1977; Culler et al., 1978; Koohmaraie et al., 1987). We observed larger ($P < .07$) MFI values at d 3, 7 and 14 from HTC LM steaks than from C LM steaks (Figure 3). Using the same species, muscle and temperature conditions, Olson et al. (1976) found that higher storage temperatures accelerated myofibril fragmentation. These authors suggested that postmortem aging effects that reduce WBS force occurred within 24 h at 25°C. Henderson et al. (1970) found that Z-line degradation occurred sooner postmortem and to a greater extent at storage temperatures of 25°C or above than at temperatures of 16°C or below.

In very rapidly chilled lean carcasses, cold shortening can occur (Lochner et al., 1980); therefore, HTC may be successful in its prevention. However, heat-toughening has been shown to occur in HTC carcasses with a high degree of finish (Lee and Ashmore, 1985). Therefore, we measured sarcomere lengths (Table 3) to check for excess shortening in either the C or HTC sides. No significant treatment difference occurred, although mean sarcomere length for each treatment was shorter than anticipated. Our findings are in agreement with those of Yu and Lee (1986) and Koohmaraie et al. (1988b), who found no HTC effects on sarcomere lengths. Glover et al. (1977) observed shorter sarcomeres at the 12th to 13th rib region in carcasses chilled at -2°C (1.73 μm) than in carcasses chilled at 3°C (1.91 μm); no WBS differences were observed. Lee and Ashmore (1985) reported sarcomere lengths of 1.66 μm for HTC LM muscles with extremely large variation, whereas the conventionally chilled LM muscles had a longer average sarcomere length (1.87 μm) with little variation. In that same study, HTC steaks were tougher from feedlot-fed cattle with 1.26 cm of fat cover than those from cattle with little fat cover, suggesting that heat-toughening had occurred.

TABLE 2. TREATMENT LEAST SQUARES MEANS (\pm SE) FOR WARNER-BRATZLER SHEAR VALUES OF LONGISSIMUS (LM) AND SEMITENDINOSUS (ST) STEAKS AND FOR SENSORY-PANEL SCORES OF LM AT 1 AND 14 DAYS POSTMORTEM

Traits	High-temp. conditioning	Control
Day 1		
ST shear force, kg	5.8 \pm .3	6.1 \pm .3
LM shear force, kg	8.3 \pm .3 ^c	9.6 \pm .3 ^d
LM juiciness ^a	4.9 \pm .1	5.1 \pm .1
LM ease of fragmentation ^a	3.8 \pm .2	3.6 \pm .2
LM connective tissue amount ^a	3.5 \pm .2	3.4 \pm .2
LM overall tenderness ^a	3.7 \pm .2	3.6 \pm .2
LM off-flavor ^b	2.6 \pm .1	2.7 \pm .1
Day 14		
ST shear force, kg	5.0 \pm .2	5.3 \pm .2
LM shear force, kg	6.9 \pm .4	7.7 \pm .4
LM juiciness ^a	5.1 \pm .1	4.8 \pm .1
LM ease of fragmentation ^a	4.6 \pm .1	4.4 \pm .1
LM connective tissue amount ^a	4.4 \pm .1	4.2 \pm .1
LM overall tenderness ^a	4.5 \pm .2	4.4 \pm .2
LM off-flavor ^b	2.5 \pm .1 ^c	2.8 \pm .1 ^d

^aA score of 6 = moderately juicy, moderately easy, traces and moderately tender; . . . 4 = slightly dry, slightly difficult, moderate and slightly tough; . . . 1 = extremely dry, extremely difficult, abundant and extremely tough.

^bA score of 4 = none; 1 = intense.

^{c,d}Means within a row without a common superscript letter differ ($P < .05$).

Because sarcomere lengths were not affected by HTC despite a difference in tenderness, HTC could be affecting the proteolytic systems involved in tenderization (Dutson, 1983), such as the cathepsin system. Cathepsins are lysosomal in nature and can degrade some myofibrillar proteins (Dutson, 1983; Ouali et al., 1984). Moeller et al. (1976) suggested that higher temperature and lower pH may disrupt the lysosome, thus releasing cathepsins. Moeller et al. (1977) found HTC to cause a decline in measurable cathepsin C in vitro activity, indicating a release of the enzyme in vivo or an increase in inhibition during the in vitro assay. In our study, cathepsin B and B + L activities were

determined at 6 h, 1 and 14 d postmortem by first isolating the lysosomes, then releasing the cathepsins by lysing the membrane. This was done to try to avoid the inhibitory reactions by cystatin as revealed by Kirschke et al. (1983), Wood et al. (1985) and Etherington et al. (1987). No treatment differences were observed for cathepsin-specific activity at any time (Table 3). Therefore, the drop in pH at the higher temperature apparently did not disrupt the lysosomal membrane by 14 d in these carcasses.

Another protease thought to be involved in postmortem tenderization is CDP. The myofibrillar proteins hydrolyzed by CDP closely mimic those proteins hydrolyzed under normal

TABLE 3. TREATMENT LEAST SQUARES MEANS (\pm SE) FOR SARCOMERE LENGTH AND CATHEPSIN B AND B + L ACTIVITY FOR THE LONGISSIMUS MUSCLE

Traits	High-temp. conditioning	Control
Sarcomere length, μ m	1.65 \pm .05	1.75 \pm .05
Cathepsin B, pmole-mg ⁻¹ .min ⁻¹		
6 h	33.5 \pm 2.8	37.6 \pm 4.3
1 d	33.1 \pm 3.0	34.3 \pm 2.9
14 d	36.5 \pm 2.1	34.7 \pm 1.9
Cathepsin B + L, pmole-mg ⁻¹ .min ⁻¹		
6 h	43.3 \pm 5.3	53.9 \pm 8.0
1 d	42.6 \pm 1.9	44.4 \pm 1.9
14 d	54.9 \pm 4.0	46.6 \pm 3.7

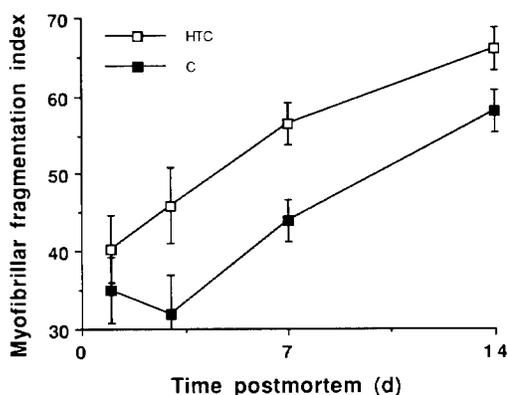


Figure 3. Longissimus myofibril fragmentation indices at 1, 3, 7 and 14 d postmortem. Bars represent SE of the mean.

postmortem conditions (Koochmarai et al., 1988a), and, unlike cathepsins, CDP is located in the cytosol (Ishiura et al., 1980). Activities of CDP-I, -II and INH were determined at 0, 6 and 24 h postmortem. Figure 4 reveals that CDP-I activity declined more rapidly in HTC muscle ($P < .01$) than in C muscle. By 6 h postmortem, 81 and 62% of initial activity (101.3 ± 5.8 units of activity/100 g of muscle) was lost in the HTC and C samples, respectively. At 24 h, additional declines of 7% and 3% were observed for HTC and C, respectively. Therefore, C muscle had more activity remaining at 24 h postmortem, which could indicate that less CDP-I was utilized in postmortem proteolysis; this could explain the less tender C LM steaks. By infusing the LM with CaCl_2 Koochmarai et al. (1989, 1990) activated the CDP system, evidenced by less CDP and INH activities at 24 h postmortem, which resulted in an improvement in LM tenderness. For CDP-II activity, a 25% decline ($P < .05$) was detected at 6 h postmortem in the HTC samples, but the 24-h assay revealed that activity was back to the initial level (97.7 ± 4.8 units of activity/100 g of muscle). This decline in CDP-II activity at 6 h was unexpected; other researchers (Vidalenc et al., 1983; Ducasting et al., 1985; Koochmarai et al., 1987) have found that postmortem storage had no effect on CDP-II activity. A significant treatment difference was observed for CDP-II activity at 24 h postmortem; however, the biological significance of this observation is questionable because the 24-h activity in the C

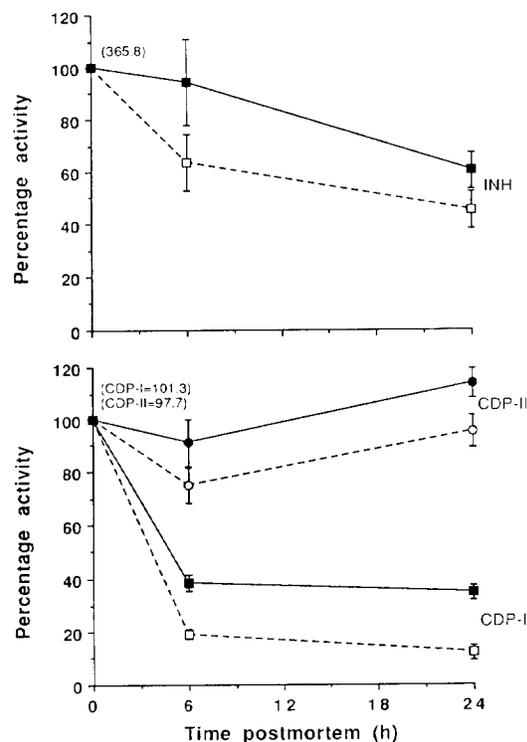


Figure 4. Longissimus calcium-dependent protease-I, -II and inhibitor percent activity remaining at 6 and 24 h postmortem. Solid line = C and dotted line = HTC. Initial activities/100 g of muscle are given in parentheses and bars represent SE of the mean.

muscle was greater than the initial activity. Inhibitor activity tended to decline faster ($P < .10$) in the HTC samples, in which 35% activity was lost by 6 h, whereas C muscles at 6 h maintained all initial INH activity (365.8 ± 24.7 units of activity/100 g of muscle). By 24 h, INH activity was higher ($P < .01$) for C muscle, with HTC and C losing 55 and 40% of their total INH activity, respectively. With conventional chilling, Koochmarai et al. (1987) reported that approximately 50% of CDP-I activity was lost by 24 h postmortem and a gradual decline was observed for 14 d; only 20% of INH activity remained at 24 h. However, their samples had been frozen, and, because INH is susceptible to freezing (Otsuka and Goll, 1987; Koochmarai, 1990), the INH values of Koochmarai et al. (1987) could have been underestimated. In the present study, assays were performed on fresh tissue for more accurate measurements of INH activity. Also, the high activity for INH could have been due



Figure 5. SDS-PAGE of myofibrillar proteins isolated from longissimus muscle of HTC and C at 0 h, 6 h, and 14 d postmortem.

to the fact that these animals were 5/8 Sahiwal. Our previous work (Whipple et al., 1990) showed that INH activity did not decline with 24 h of postmortem storage in Sahiwal crosses, as it did with *Bos taurus* crosses.

Myofibrillar protein SDS-PAGE failed to reveal any treatment differences in protein degradation at 6 h, 1 and 14 d postmortem (Figure 5). However, it did indicate that proteolysis had occurred by 6 h postmortem for both treatments. Whipple et al. (1990) observed differences in postmortem proteolysis between Hereford \times Angus crosses and 5/8 Sahiwal crosses. For 5/8 Sahiwals in that study, desmin remained at d 14 and a 30,000-dalton component failed to appear. Therefore, the high WBS values for both C and HTC steaks from 5/8 Sahiwals probably were due to a lack of myofibrillar proteolysis. In most cases with 14 d of storage, a 30,000-dalton component has appeared, and desmin has been degraded (MacBride and Parrish, 1977; Koohmaraie et al., 1984).

These results indicate that HTC marginally improved tenderness of LM steaks from 5/8 Sahiwal crosses and, of all the traits measured, only CDP and INH were affected by this treatment. Therefore, CDP-I and(or) INH probably play a primary role in the postmortem tenderization of meat.

Implications

The application of high-temperature conditioning to 5/8 Sahiwal crossbred carcasses improved tenderness of longissimus steaks at d 1 postmortem. High-temperature conditioning also affected the activities of calcium-dependent protease-I and its inhibitor, which suggests that postmortem tenderization is related to their activities. The improvement in tenderness with high-temperature conditioning still was not sufficient for any 5/8 Sahiwal steaks to have acceptable shear force values (< 5.0 kg); therefore, other techniques besides high-temperature conditioning must be utilized. In addition, the feasibility of high-temperature conditioning is questionable due to microbial growth concerns.

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