EFFECTS OF HYDRODYNAMIC PRESSURE PROCESSING AND BLADE TENDERIZATION ON INTRAMUSCULAR COLLAGEN AND TENDERNESS-RELATED PROTEIN CHARACTERISTICS OF TOP ROUNDS FROM BRAHMAN CATTLE*

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ABSTRACT

This study evaluated the effects of blade tenderization (BT), hydrodynamic pressure processing (HDP) and BT followed by HDP (BT + HDP) on collagen solubility and tenderness-related protein characteristics in top rounds from Brahman cattle. Top rounds ($n = 12$*) were divided in half and randomly assigned to HDP, BT or BT + HDP with each treatment sample having a paired control. HDP and BT + HDP increased (*$P < 0.0001$*) collagen solubility, but the correlation between percent soluble collagen and tenderness was low (*$r = -0.41$*). All three treatments increased (*$P < 0.01$*) fragmentation of myofibrils 35% compared with controls. HDP and BT + HDP treatments increased (*$P < 0.05$*) the content of a 100 to 110-kDa protein observed in myofibrillar protein fractions by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Minimal treatment differences were observed in sarcoplasmic proteins using SDS-PAGE. Results suggest BT physically disrupts the muscle structure to improve tenderness while HDP tenderization potentially results from both physical disruption of the muscle structure and some form of direct alterations to muscle proteins.*

* Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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INTRODUCTION

Inconsistent beef tenderness is a major problem in the meat industry. With the high consumer demand for lean yet tender cuts of beef and the high degree of tenderness variability between muscles and animals, the development of postharvest techniques to tenderize tough cuts of beef would be of great benefit to the meat industry. Meat tenderness is primarily a function of the "actomyosin toughness" due to the myofibrillar proteins (Locker 1960; Marsh and Leet 1966) and the "background toughness" due to the connective tissue (Bailey 1972). The ability of postharvest techniques to enhance tenderness is likely a product of their effect on these two components of tenderness. Blade tenderization (BT) is a postharvest technique that has been shown to effectively tenderize meat by using blade or needle probes to disrupt the myofibrillar apparatus and connective tissue (Seideman *et al*. 1977; Boyd *et al*. 1978; Savell *et al*. 1982; Jeremiah *et al*. 1999). Hydrodynamic pressure processing (HDP) is a novel technique that utilizes shock waves in water to tenderize meat products (Solomon *et al*. 1997). HDP has been shown to reduce shear force values in fresh and cold-shortened beef by 30 to 72% depending upon the muscle and HDP parameters (Solomon *et al*. 1997). Previously, Liu *et al.* (2006) investigated the utilization of BT and HDP alone, and in combination to tenderize beef top rounds from Brahman cattle, which have inherent tenderness problems (Crouse *et al*. 1989). Although previous work has established that BT and HDP technologies effectively tenderize meat, there is a lack of data characterizing the effects these technologies have on myofibrillar and stromal proteins as they relate to muscle ultrastructure and the mechanism of tenderization. Thus, the objective of this study was to investigate the effects of BT, HDP and BT followed by HDP (BT + HDP) on protein characteristics related to tenderness and muscle protein functionality.

MATERIALS AND METHODS

Muscle Samples

Thirty-two top rounds from Brahman market cattle (U.S. Department of Agriculture, SubTropical Agriculture Research Station, Brooksville, FL) were removed from carcasses, vacuum packaged, frozen at 7 days postmortem and shipped to the Food Technology and Safety Laboratory, Beltsville, MD. Prior to the experiment, one 2.5-cm-thick steak was removed from the proximal end of each frozen top round for the determination of Warner-Bratzler shear (WBS) force values (AMSA 1995). The 12 top rounds selected for use in this experiment were considered "very tough," having shear force values ranging from 6.8 to 9.8 kgf and were part of a larger study (Liu *et al*. 2006).

Experimental Design

The experimental design used in this study is detailed in Liu *et al*. (2006). The treatments used in this experiment were as follows: BT, HDP, BT + HDP and untreated controls. In order to prevent animal-to-animal variation from confounding treatment comparisons, ideally each round would be divided into four sections and each of the three treatments and the control would be randomly assigned. However, because of significant intramuscular shear force differences within the *semimembranosus* (Reuter *et al*. 2002) and the size of the top rounds, it was not possible to obtain all three treatments and the control from each top round. Thus, a balanced incomplete block design was constructed to minimize and balance the effects of known inter- and intra-animal variations on treatment differences. A complete replicate of treatment application was accomplished by applying each of the three possible pairings of the three treatments: (BT, HDP), (BT, BT + HDP) and (HDP, BT + HDP) to a separate top round (i.e., incomplete block) during the same processing day with each treatment assigned to two of the three top rounds comprising the replicate. Top rounds (i.e., blocks) were divided into two sections, parallel to the long axis of the *semimembranosus* muscle (from the origin to insertion end), and a treatment was applied to each section. Each treatment section was divided to remove a subsection (approximately 5.5-cm thick) perpendicular to the long axis of the muscles prior to treatment to serve as the paired control. A total of four replicates were conducted to allow each treatment and its paired control to be assigned once to each of the four different locations within a top round (proximal to distal and medial to deep side).

Sample Preparation

Three frozen top rounds were thawed at 2C for 96–120 h for each processing day. The *gracilis* muscle was removed from each top round on the day of treatment (day 0). Following treatment application, two 2.5-cm steaks were removed from each treated and paired control section and randomly designated for day 0 and day 7 tenderness evaluation. The methodologies and data for cooking and shear force are described in Liu *et al*. (2006). After treatment on day 0, 2.5-cm-thick steaks were removed from each treated and paired control section, vacuum packaged and frozen for subsequent analyses of collagen and protein characteristics. Muscle samples for collagen and protein data were only collected on day 0 and not day 7 because of the size of the top rounds.

Treatments

Because of the thickness of the top rounds, BT and BT + HDP samples were passed one time through the blade tenderizer unit (2.3 penetrations/cm^2)

(Model MT-M5, Lumar Ideal Inc., Montreal Quebec, Canada) parallel to the muscle fibers. Two randomly selected HDP and/or BT + HDP samples were packaged in a single Cryovac boneguard bag (Model 620TBGW, Sealed Air Corp., Duncan, SC), heat shrunk (97C) and placed onto a 1.3-cm-thick flat metal plate inside a 98-L plastic explosive container filled with water. The container was suspended 25 cm above the floor and a 100-g cylindrical shaped binary explosive was detonated 31 cm above the meat to generate the HDP shock wave.

Myofibrillar Fragmentation Index (MFI)

MFI was determined on triplicate 0.5-g samples as described by Hopkins *et al*. (2000) with modifications. Muscle samples were combined with 30 mL of ice-cold MFI buffer (0.1-M KCl, 1-mM ethylenediaminetetraacetic acid, 1-mM NaN_3, 25-mM potassium phosphate [7-mM KH_2PO_4 and 18-mM K_2HPO_4 giving a buffer pH of 7.0 at 4–5C]) in a 400-mL homogenization chamber. Samples were homogenized twice for 30 s at 15,000 rpm (speed setting #10 on Omni Mixer; Ivan Sorvall Inc., Norwalk, CT) with a 30-s interval between homogenizations. The myofibril suspension was then filtered through a mesh strainer (1-mm^2 holes) to remove the connective tissue, and the residual myofibrils in the mesh were washed through with 10 mL of buffer. The suspension was centrifuged for 10 min at 1000 × g (2C) and the supernatant decanted. The myofibril pellet was suspended in 10 mL of buffer by vortex mixer and centrifuged for 10 min at 1000 × g (2C), after which the supernatant was discarded. The resuspension/centrifugation process was repeated twice more, and the final pellet was suspended in 10 mL of buffer. The protein concentration was determined using the biuret method (Gornall *et al*. 1949), and aliquots were diluted with buffer to 0.5 mg/mL in triplicate. The absorbance at 540 nm of each 0.5 mg/mL suspension was then immediately measured using a spectrophotometer (UV-2501PC, Shimadzu Corp., Columbia, MO), and the mean of the triplicate absorbance readings was multiplied by 150 to give the index value for MFI.

Collagen Amount and Solubility

Frozen samples for intramuscular collagen determination were tempered at 4C, trimmed of fat and epimysial connective tissue and cut into approximate 7 × 7 × 7-mm cubes. Samples were then chopped in a food processor (Handy-Chopper Plus HC3000, Black and Decker, Hamstead, MD) for three cycles of 10 s on and 10 s off to obtain a fine homogenate without complete thawing. Chopped samples were then stored at –20C until further analysis.

Collagen assays were performed in triplicate. Total collagen samples were weighed directly into a hydrolysis tube. The soluble collagen was

separated from the insoluble residue according to the procedure of Hill (1966). The collagen was solubilized by heating 3–5 g of sample in 20 mL of 1:3 Ringer's solution (147-mM NaCl, 4-mM KCl, 3-mM $CaCl_2$) at 77C for 70 min in a circulating water bath (Lauda E106T, Brinkman Instruments, Inc., Westbury, NY). After centrifuging for 10 min at 4330 × g, the supernatant was decanted to a hydrolysis tube. The pellet was resuspended in 10 mL of dilute 1:3 Ringer's solution and centrifuged as described earlier. The supernatants containing the soluble collagen were pooled, and the pellet (insoluble residue fraction) was transferred to another hydrolysis tube. Samples were hydrolyzed in 6-M HCl using a microwave digestion system (MDS2000; CEM Corp., Matthews, NC) as outlined by Bauer (1991). Digestion was conducted for 30 min at 85 psi pressure with the following schedule: 100% power to pressure limit of 40 psi, 100% power to 85 psi for 30 min and 50% power to pressure of 40 psi. Temperature and pressure histories were monitored to verify digestion. The hydrosylates were quantitatively transferred to a flask and brought to 500-mL volume with deionized water and mixed well. A 50-mL aliquot was filtered and reserved for hydroxyproline analysis. Collagen amount was determined from a hydroxyproline standard curve following color development according to AOAC procedure 990.26G (AOAC 1990) except that the acetate–citrate buffer of Stegman and Stalder (1967) was used. Briefly, a 1-mL aliquot of hydrosylate was oxidized for 20 ± 2 min using Chloramine-T solution. Addition of 2 mL of p-dimethylaminobenzaldehyde in perchloric acid solution stopped the oxidation, and the red pyrrol color was developed for 5 min in a water bath at 90C. Samples were cooled to room temperature and absorbance at 558 nm was measured within 45 min using a spectrophotometer with sipper attachment (Model 1650, Shimadzu Corp., Columbia, MD). Hydroxyproline concentration was converted to collagen using factors of 7.25 for soluble fraction (Goll *et al*. 1963) and 7.52 for total and insoluble residue fractions (Cross *et al*. 1973). Soluble collagen percentage was calculated based on the amount solubilized per total amount.

Protein Solubility

Protein solubility was measured using the procedure of Schilling *et al*. (2002) with modifications. Sarcoplasmic solubility was determined by homogenizing duplicate 1-g muscle samples with 10 mL of ice-cold 0.025-M potassium phosphate buffer (pH 7.2) in four 4-s bursts with a Kinematica polytron (Model PT 10/35, Brinkman Instruments, Inc.). The homogenate was incubated overnight at 4C and then centrifuged at 2600 × g for 30 min. The supernatant was decanted and protein concentration was measured using the biuret method. Total solubility was similarly determined in 1.0-M KI/0.1-M potassium phosphate (pH 7.2) buffer. Myofibrillar protein solubility was calculated from the difference between total and sarcoplasmic protein solubility.

Protein Isolation

Myofibrillar and sarcoplasmic protein fractions were isolated according to the procedures of Doerscher *et al*. (2004) and Goll *et al*. (1974) with modifications. Trimmed muscle samples were first minced for 5 s in a handheld blender (Braun MR 370, Braun, Inc., Woburn, MA). Approximately 10 g of minced muscle was homogenized with 90 mL of ice-cold standard salt solution (SSS) (100-mM KCl, 20-mM K_2HPO_4/KH_2PO_4, pH 7.0, 2-mM $MgCl_2$, 1-mM EGTA, 1-mM NaN_3) using a Waring Blendor equipped with a rheostat (Powerstat, Superior Electric Co., Bristol, CT) at a setting of 65% for 10 s. The total homogenate was then filtered through two layers of cheesecloth. A 10-mL aliquot was removed from each sample, and the remaining homogenate was centrifuged at 1500 × g for 20 min (4C). The supernatant was decanted, and centrifuged at 10,000 × g for 30 min. After filtering through two layers of cheesecloth, the supernatant was saved as the sarcoplasmic protein fraction.

The myofibrillar pellet resulting from the initial centrifugation of the total homogenate was resuspended in 20 mL of SSS, homogenized using three 4-s bursts with a Kinematica polytron and centrifuged at 1000 × g for 10 min. The pellet was then resuspended, homogenized and centrifuged twice more using SSS. The pellet was then resuspended and washed two times in SSS + 1% Triton X-100 (Sigma-Aldrich Co., St. Louis, MO) and centrifuged at 1500 × g for 10 min. The pellet was then resuspended and washed two additional times in 15 mL of SSS and centrifuged at 1500 × g for 10 min. The washed myofibril pellet was then resuspended in 5 mL of a solution containing 50% (v/v) buffer (100-mM KCl, 5-mM Tris, pH 7.0) and 50% (v/v) glycerol.

Gel Sample Preparation and Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) Analysis

The protein concentrations of isolated protein fractions were determined using the biuret method and the concentrations were adjusted to 4 mg/mL for sarcoplasmic samples and 1.6 mg/mL for myofibril samples. One volume of each sample was then mixed with one volume of sample buffer/tracking dye solution (8-M urea, 2-M thiourea, 0.05-M tris[hydroxymethyl]aminomethane [Tris, pH 6.8], 75-mM dithiothreitol, 3% SDS, 0.05% bromophenol blue), heated in boiling water for 5 min, cooled on ice and stored at −80C until analysis. Myofibrillar samples were loaded (8 μg/lane) onto precast 4–20% acrylamide gradient Tris-HCl gels. Sarcoplasmic samples were loaded (30 μg/lane) onto precast 10–20% acrylamide gradient Tris-HCl gels. The gels were run in duplicate on the Bio-Rad Criterion gel system (Bio-Rad Laboratories, Hercules, CA) at a constant voltage (200 V) for approximately 1 h at 4C. The running buffer was 25-mM Tris (pH 8.3), 192-mM glycine and 0.1% SDS (w/v). Gels were stained in a solution of 0.05% Coomassie brilliant blue R-250

(Bio-Rad Laboratories, Hercules, CA), 40% (v/v) methanol and 7% (v/v) glacial acetic acid, and destained in 15% (v/v) methanol and 7% (v/v) glacial acetic acid. Gel images were captured using an imaging system (KODAK Gel Logic 200, Eastman KODAK Co., Rochester, NY), and KODAK 1D Image Analysis software was used to measure the relative staining intensity of protein bands.

Statistical Analysis

Collagen, MFI, protein solubility and SDS-PAGE band intensity data were analyzed as a two-way analysis of variance using the PROC MIXED procedure of SAS (version 9.1, SAS Institute Inc., Cary, NC, 2002–2003). The model included treatment group (control versus treated), treatment (BT, HDP or BT + HDP), and treatment × group interaction as fixed effects and top round within trial as a random block effect. Significant differences ($P < 0.05$) between means were identified using least significant difference pairwise *t*-tests. Correlation coefficients were determined using the PROC CORR procedure.

RESULTS

Intramuscular Collagen Amount and Solubility

The effects of the tenderization treatments on the total, insoluble and soluble collagen are shown in Table 1. No significant differences were found for the total amount of collagen in the top round steaks. Significant treatment × group interactions, however, were noted for the amounts of insoluble and soluble collagen. Compared with controls, BT decreased ($P < 0.05$) insoluble collagen 16.8%, while BT + HDP increased ($P < 0.05$) residual collagen 26.5% and HDP alone had no significant effect. HDP and BT + HDP treatments increased ($P < 0.05$) the amount of soluble collagen compared with controls 47.8 and 54.4%, respectively, while BT had no significant effect. Similar results were obtained when the amount of soluble collagen was expressed as a percentage of the total collagen. Compared with controls, HDP and BT + HDP increased ($P < 0.05$) the percentage of soluble collagen 45.6 and 33.3%, respectively, while BT had no significant effect.

MFI and Protein Solubility

The effects of the tenderization treatments on MFI and protein solubility characteristics are shown in Table 2. BT-, HDP- and BT + HDP-treated samples had 35% higher ($P < 0.01$) MFI values than paired controls. MFI values did not differ among the three treatments, however. Although treated

TABLE 1.
INTRAMUSCULAR COLLAGEN AMOUNT AND SOLUBILITY FOR BT, HDP, BT + HDP AND PAIRED CONTROL SAMPLES FROM BRAHMAN TOP ROUNDS (LEAST SQUARE MEANS AND STANDARD ERRORS)

Treatment	Group		Level of significance*		
	Control	Treated	Treatment	Group	T × G
Total collagen (μg/g dry basis)					
BT	593.3 (32.4)	577.6 (33.2)	NS	NS	NS
HDP	512.0 (36.8)	543.3 (31.8)			
BT + HDP	526.9 (25.6)	599.8 (32.2)			
Insoluble collagen (μg/g dry basis)					
BT	576.0^{b} (36.9)	479.3^{c} (37.0)	0.0001	NS	0.0001
HDP	467.4^{c} (39.8)	510.2^{bc} (35.9)			
BT + HDP	526.8^{bc} (35.3)	666.6^{a} (35.7)			
Soluble collagen (μg/g dry basis)					
BT	40.1^{bc} (4.4)	38.7^{bc} (4.4)	0.0174	0.0001	0.0001
HDP	29.5^{d} (4.5)	43.6^{b} (4.3)			
BT + HDP	35.3^{cd} (4.2)	54.5^{a} (5.7)			
Soluble collagen %					
BT	6.49^{b} (0.65)	6.90^{b} (0.65)	0.0266	0.0001	0.0002
HDP	5.53^{c} (0.67)	8.05^{a} (0.64)			
BT + HDP	6.48^{b} (0.64)	8.64^{a} (0.73)			

[a–d] Least square means with different superscripts differ significantly ($P < 0.05$).
* NS indicates not significant ($P > 0.05$).
BT, blade tenderization; HDP, hydrodynamic pressure processing; BT + HDP, combination treatment of blade tenderized followed by hydrodynamic pressure processing.

samples consistently had numerically lower sarcoplasmic protein solubility than paired controls, these differences were not significant ($P > 0.05$). Neither myofibrillar nor total protein solubility differed statistically ($P > 0.05$) between treated and control samples. Additionally, myofibrillar and total protein solubility did not differ among treatments. It should be noted, however, that HDP- and BT + HDP-treated samples had higher myofibrillar protein solubility than paired controls in all but two of the top rounds. When myofibrillar protein solubility data were analyzed without these two samples, HDP- and BT + HDP-treated samples were approximately 30% higher than control samples while BT samples did not differ from controls (data not shown). With the outliers removed, the total protein solubility was approximately 19% higher in HDP- and BT + HDP-treated samples compared with controls.

SDS-PAGE Profiles of Myofibrillar and Sarcoplasmic Proteins

Figure 1 displays an example of the electrophoretic profiles of the myofibrillar protein fractions from BT-, HDP- and BT + HDP-treated samples and

TABLE 2.
MYOFIBRILLAR FRAGMENTATION INDEX (MFI) AND PROTEIN SOLUBILITY OF BT, HDP, BT + HDP AND PAIRED CONTROL SAMPLES FROM BRAHMAN TOP ROUNDS (LEAST SQUARE MEANS AND STANDARD ERRORS)

Treatment	Group		Level of significance*		
	Control	Treated	Treatment	Group	T × G
MFI					
BT	45.8[b] (6.3)	62.2[a] (8.2)	NS	0.0020	NS
HDP	46.5[b] (7.0)	62.7[a] (6.9)			
BT + HDP	45.8[b] (6.7)	61.4[a] (5.9)			
Sarcoplasmic protein solubility (mg protein/g muscle tissue)					
BT	41.2 (2.9)	38.3 (2.1)	NS	NS	NS
HDP	42.2 (3.9)	41.9 (2.1)			
BT + HDP	44.3 (2.9)	40.6 (1.8)			
Myofibrillar protein solubility (mg protein/g muscle tissue)					
BT	110.9 (13.6)	109.9 (13.5)	NS	NS	NS
HDP	134.3 (13.6)	127.4 (12.8)			
BT + HDP	111.3 (9.1)	127.1 (11.1)			
Total protein solubility (mg protein/g muscle tissue)					
BT	153.0 (15.1)	148.4 (13.6)	NS	NS	NS
HDP	177.1 (14.3)	169.1 (12.8)			
BT + HDP	154.7 (9.3)	167.1 (11.5)			

[a,b] Least square means with different superscripts differ significantly ($P < 0.05$).
* NS indicates not significant ($P > 0.05$).
BT, blade tenderization; HDP, hydrodynamic pressure processing; BT + HDP, combination treatment of blade tenderized followed by hydrodynamic pressure processing.

their controls, and the relative intensities of the prominent myofibrillar protein bands are shown in Table 3. As a percentage of the total staining intensity within each lane, the intensity of the actin band varied minimally from sample to sample. Thus, the measured intensity of each band was expressed as the ratio of the intensity of the band of interest to the intensity of the actin band in order to account for any errors in protein loading on the gels. Although there existed considerable variation in the profiles of the myofibrillar fractions among top rounds, visually there were no consistent large scale shifts in the banding patterns between control and treated samples (Fig. 1). The tenderization treatments, however, influenced the relative abundance of the 100 to 110-kDa band (Table 3). Compared with their paired controls, HDP- and BT + HDP-treated samples increased ($P < 0.05$) the concentration of the 100 to 110-kDa band 16 and 21%, respectively. BT treatment alone showed no significant effect. No significant treatment or group effects were observed in desmin (55 kDa) or troponin-T (38 kDa) bands. The 30-kDa protein fragment exhibited a significant treatment effect ($P < 0.05$) with HDP samples having a

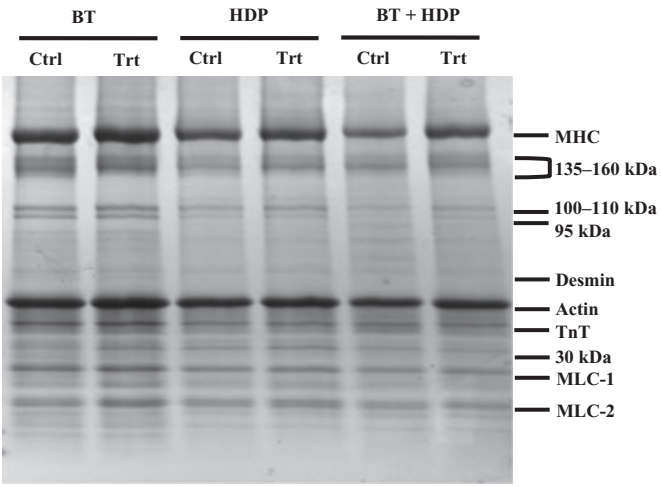

FIG. 1. SODIUM DODECYL SULFATE POLYACRYLAMIDE GEL ELECTROPHORESIS PROFILES OF MYOFIBRILLAR PROTEINS FROM BLADE TENDERIZATION (BT), HYDRODYNAMIC PRESSURE PROCESSING (HDP), BT + HDP AND PAIRED CONTROL SAMPLES FROM BRAHMAN TOP ROUNDS
Ctrl, control; Trt, treated; MHC, myosin heavy chain; MLC, myosin light chain; TnT, troponin-T.

higher concentration than BT samples, with BT + HDP samples intermediate. Myosin heavy chain, 135–160 kDa, 95 kDa and myosin light chain (MLC) bands showed no significant effects.

Figure 2 displays an example of the electrophoretic profiles of the sarcoplasmic protein fractions from BT-, HDP- and BT + HDP-treated samples and their paired controls. The abundance of individual protein bands relative to the total protein per lane varied widely among top rounds, particularly for 195-, 145-, 90-, 63-, 42-, 36-, 29-, 25- and 14-kDa bands. Minimal differences were found, however, between paired control and treated samples. Likewise, few differences were observed among BT, HDP and BT + HDP treatments with respect to sarcoplasmic protein banding patterns.

Correlation of Parameters with Tenderness Measurements

Table 4 shows the simple correlation coefficients of the collagen and tenderness measurements. As expected, day 0 and day 7 postmortem tenderness measurements were strongly correlated (0.73), and the amount of insoluble residue and soluble collagen was correlated ($P < 0.001$) to the total amount of collagen, 0.61 and 0.56, respectively. Overall, the various collagen parameters were not highly correlated to tenderness measurements (r-values

TABLE 3.
CONTENT† OF MAJOR MYOFIBRILLAR PROTEINS OF BT, HDP, BT + HDP AND PAIRED CONTROL SAMPLES FROM BRAHMAN TOP ROUNDS (LEAST SQUARE MEANS AND STANDARD ERRORS)

Treatment	Group		Level of significance*		
	Control	Treated	Treatment	Group	T × G
Myosin heavy chain					
BT	1.470 (0.083)	1.440 (0.077)	NS	NS	NS
HDP	1.445 (0.089)	1.505 (0.080)			
BT + HDP	1.482 (0.094)	1.509 (0.084)			
135 to 160-kDa band					
BT	0.566 (0.051)	0.542 (0.045)	NS	NS	NS
HDP	0.523 (0.059)	0.570 (0.045)			
BT + HDP	0.546 (0.059)	0.567 (0.049)			
100 to 110-kDa band					
BT	0.245[b] (0.020)	0.256[ab] (0.028)	NS	0.0153	NS
HDP	0.250[b] (0.018)	0.290[a] (0.019)			
BT + HDP	0.239[b] (0.018)	0.289[a] (0.020)			
95-kDa band					
BT	0.157 (0.021)	0.148 (0.020)	NS	NS	NS
HDP	0.194 (0.021)	0.166 (0.018)			
BT + HDP	0.162 (0.021)	0.162 (0.018)			
Desmin					
BT	0.139 (0.028)	0.125 (0.022)	NS	NS	NS
HDP	0.169 (0.027)	0.164 (0.026)			
BT + HDP	0.155 (0.022)	0.151 (0.024)			
Troponin-T					
BT	0.615 (0.058)	0.582 (0.061)	NS	NS	NS
HDP	0.734 (0.065)	0.599 (0.065)			
BT + HDP	0.614 (0.065)	0.720 (0.069)			
30-kDa band					
BT	0.193[c] (0.012)	0.202[bc] (0.016)	0.0457	NS	NS
HDP	0.228[ab] (0.017)	0.236[a] (0.013)			
BT + HDP	0.208[bc] (0.012)	0.219[ab] (0.011)			
MLC-1					
BT	0.253 (0.018)	0.249 (0.018)	NS	NS	NS
HDP	0.287 (0.020)	0.265 (0.019)			
BT + HDP	0.251 (0.020)	0.231 (0.019)			
MLC-2					
BT	0.387 (0.030)	0.343 (0.028)	NS	NS	NS
HDP	0.392 (0.031)	0.410 (0.028)			
BT + HDP	0.399 (0.030)	0.375 (0.028)			

[a–d] Least square means with different superscripts differ significantly ($P < 0.05$).
* NS indicates not significant ($P > 0.05$).
† Content expressed as the intensity ratio between the band of interest and actin.
BT, blade tenderization; HDP, hydrodynamic pressure processing; BT + HDP, combination treatment of blade tenderized followed by hydrodynamic pressure processing; MLC, myosin light chain.

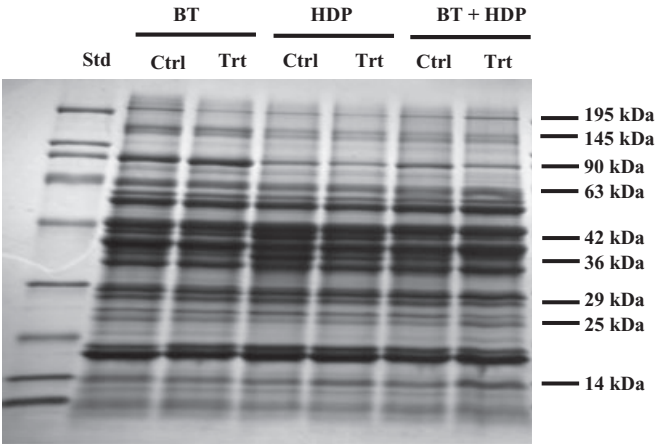

FIG. 2. SODIUM DODECYL SULFATE POLYACRYLAMIDE GEL ELECTROPHORESIS PROFILES OF SARCOPLASMIC PROTEINS FROM BLADE TENDERIZATION (BT), HYDRODYNAMIC PRESSURE PROCESSING (HDP), BT + HDP AND PAIRED CONTROL SAMPLES FROM BRAHMAN TOP ROUNDS
Ctrl, control; Trt, treated; Std, molecular weight standard (WBS).

TABLE 4.
CORRELATION COEFFICIENTS (r) BETWEEN COLLAGEN AND TENDERNESS MEASUREMENTS OF BRAHMAN TOP ROUNDS

Parameter	1	2	3	4	5	6
1. Day 0 WBS	1.00	0.73***	–0.31*	–0.39**	–0.50***	–0.41**
2. Day 7 WBS		1.00	–0.33*	–0.42**	–0.47**	–0.37*
3. Total collagen			1.00	0.61***	0.56***	0.16**
4. Residual collagen				1.00	0.57***	0.30***
5. Soluble collagen					1.00	0.87***
6. Soluble collagen %						1.00

Levels of significance: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

–0.31 to –0.50), with only minor differences in the collagen correlations with day 0 and day 7 tenderness. The amount of soluble collagen had the highest correlation with tenderness (–0.50 and –0.47), total collagen had the lowest correlation with tenderness (–0.31 and –0.33) and insoluble collagen was intermediate.

Correlations between the various protein parameters, SDS-PAGE profiles and tenderness measurements are shown in Table 5. MFI was strongly correlated ($P < 0.001$) with tenderness on day 0 and day 7 (–0.57 and –0.62, respectively). Solubility parameters were not significantly correlated to

TABLE 5.
CORRELATION COEFFICIENTS (r) BETWEEN TENDERNESS (WBS), MYOFIBRILLAR FRAGMENTATION INDEX (MFI), PROTEIN SOLUBILITY AND SODIUM DODECYL SULFATE POLYACRYLAMIDE GEL ELECTROPHORESIS PROTEIN PROFILE MEASUREMENTS OF BRAHMAN TOP ROUNDS

Parameter	Day 0 WBS	Day 7 WBS	MFI
MFI	–0.57***	–0.62***	
Sarcoplasmic protein solubility	–0.15	–0.21	0.03
Myofibrillar protein solubility	–0.06	–0.21	0.12
Total protein solubility	–0.09	–0.24	0.11
Myofibrillar proteins:			
Myosin heavy chain	–0.45**	–0.39*	0.36*
135 to 160-kDa band	0.23	0.28	–0.23
100 to 110-kDa band	–0.13	–0.06	0.33*
95-kDa band	–0.26	–0.25	0.42**
Desmin	0.09	0.09	–0.13
Troponin-T	0.22	0.36	–0.32*
30-kDa band	–0.02	–0.05	0.38*
MLC-1	0.31*	0.30*	–0.20
MLC-2	0.17	0.02	–0.22
Sarcoplasmic proteins (kDa band)			
195	–0.13	–0.25	0.07
145	–0.22	–0.34*	0.39**
90	–0.42**	–0.43**	0.39**
63	–0.25	–0.28	0.18
42	–0.02	–0.03	0.13
36	0.02	–0.15	–0.08
29	0.30*	0.28	–0.02
25	0.15	0.15	–0.44**
14	0.34*	0.47**	–0.35*

Levels of significance: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.
MLC, myosin light chain.

tenderness. In general, correlations between tenderness and the composition of the myofibrillar protein fractions were low. Only the relative abundance of myosin heavy chain and MLC-1 correlated ($P < 0.05$) with day 0 or day 7 tenderness measurements. MFI was significantly correlated to the relative abundance of myosin heavy chain (0.36), the 100 to 110-kDa protein band (0.33), the 95-kDa protein band (0.42), troponin-T (–0.32) and the 30-kDa protein band (0.38). Similarly, sarcoplasmic protein profiles demonstrated an overall low correlation with tenderness as only the 145-, 90-, 29-, 25- and 14-kDa protein bands were correlated ($P < 0.05$) with MFI or WBS measurements.

DISCUSSION

The integrity and composition of the myofibrillar contractile apparatus and the intramuscular connective tissue play key roles in determining meat tenderness (Locker 1960; Marsh and Leet 1966; Bailey 1972). The purpose of the current study was to determine the effects that postharvest techniques, BT and HDP, have on these two components of tenderness. BT is thought to tenderize meat by the mechanical severance of both the myofibrillar structure and connective tissue (Boyd *et al*. 1978; Jeremiah *et al*. 1999). The tenderizing effect of HDP is thought to be related to its ability to rupture sarcomeres at the Z-line and A-band/I-band juncture (Zuckerman and Solomon 1998). When HDP and BT were combined, it was hypothesized that the disruption of the connective tissue and myofibrillar structure by BT would enhance the tenderizing effect of HDP. However, no synergistic treatment effect on tenderness was observed in the shear force data of these samples, which were reported by Liu *et al*. (2006). Tenderness improved 18% with BT and HDP, and 14% with BT + HDP on day 0. Additionally, with HDP and BT + HDP treatments tenderness increased between day 0 and day 7, whereas tenderness did not change with aging in BT-treated top rounds.

Collagen is the predominant protein in endomysial and perimysial connective tissue (Dransfield 1977). This connective tissue component of beef tenderness is primarily influenced by the content and solubility of collagen (Cross *et al*. 1973). Decreased collagen content and increased collagen solubility are generally thought to positively impact beef tenderness (Berry *et al*. 1974; Light *et al*. 1985). As a result, postharvest techniques that disrupt connective tissue and/or increase collagen solubility may enhance tenderness. While the BT treatment in the current study may have contributed to tenderization by physically severing the connective tissue, BT treatment had no direct effect on the total amount or solubility of collagen (Table 1). This finding supports past data on the effects of BT on sensory panel evaluations of connective tissue. Schilling *et al*. (2003) observed no differences between the sensory-evaluated amount of connective tissue in BT-treated and control steaks from U.S. Utility grade cows, which would most likely have a high proportion of cross-linked (i.e., less soluble) collagen. Studies conducted on muscles obtained from young cattle have shown that the amount of connective tissue perceptible to a sensory panel is either not affected or slightly decreased with BT treatments, depending on the specific muscle (Wheeler *et al*. 1990; Jeremiah *et al*. 1999; George-Evins *et al*. 2004).

Studies have shown varying effects of HDP on collagen characteristics. Using scanning electron microscopy, Zuckerman *et al*. (1999) showed that endomysial connective tissue was slightly disrupted in HDP-treated beef *semimembranosus*, leading to the hypothesis that HDP may increase collagen

solubility. This was supported by the current study in which the HDP and BT + HDP treatments increased the amount of soluble collagen measured, indicating that HDP enhances connective tissue solubility (Table 1). Marriott *et al*. (2001), however, did not observe differences between HDP-treated and controls in regard to soluble, insoluble or total collagen content. Varying results between the current study and that of Marriott *et al*. (2001) are likely due to differences in the age of the animals utilized, muscles studied and parameters of the HDP treatment. Marriott *et al*. (2001) used *longissimus* samples from U.S. Commercial and Utility grade cow carcasses and demonstrated only minimal reductions in shear force with HDP treatment (controls: 3.21–4.49 kgf versus treated: 2.90–3.83 kgf).

Data from this study suggest that the tenderizing effects of HDP and BT on the top rounds were not closely related to changes in total and insoluble collagen content. As expected, total collagen content varied greatly between samples but was not influenced by the tenderization treatments. Further investigation is needed to explain the observation that residual (insoluble) collagen content decreased with BT treatment and increased with BT + HDP treatment. Neither total nor residual collagen content, however, was highly correlated to shear force values ($r = -0.31$ to -0.42). While HDP and BT + HDP increased soluble collagen content approximately 50%, this probably did not play a major role in the tenderization process because the proportion of soluble collagen was low (5.5 to 8.6% of total collagen), and the relationship between tenderness and collagen solubility was weak ($r = -0.37$ to -0.41). HDP effects on collagen solubility may have a stronger impact on tenderness in muscles with a higher proportion of soluble collagen. Overall, the low correlation between collagen characteristics and shear force values in this study seems to indicate that BT and HDP treatments probably acted more directly on the myofibrillar components of tenderness.

The degree of sarcomere shortening, integrity of the myofibrillar ultrastructure and intermyofibril linkages are key factors in the nonconnective tissue component of tenderness. Sarcomere length was not expected to be influenced by HDP or BT treatments and was not measured. The MFI, which is a measure of the extent of fragmentation of myofibrils caused by homogenization, is highly correlated to meat tenderness (Culler *et al*. 1978). Taylor *et al*. (1995) demonstrated that MFI was a useful indicator of the extent of proteolysis, reflecting the degradation of both structural proteins within the I-band of the sarcomere and intermyofibril linkages. Thus, the observed increase in MFI in treated samples and the significant correlation between MFI and shear force values suggest that the tenderizing effects of HDP and BT are partially the result of physical disruptions of the myofibrillar ultrastructure and intermyofibril linkages. Similar increases in MFI values are observed with the proteolytic breakdown of proteins as meat ages (Taylor *et al*. 1995).

While BT tenderizes muscle by mechanical severance, HDP shock waves may tenderize meat by directly altering muscle proteins in addition to physically disrupting the structure. This is supported by data showing that as HDP-treated beef becomes more tender, there is a redistribution of proteins between the myofibrillar and sarcoplasmic fractions (Spanier and Romanowski 2000). A possible explanation for this redistribution may be HDP induced changes in protein solubility and protein degradation. Thus, the solubility and SDS-PAGE profiles of myofibrillar and sarcoplasmic protein fractions were investigated in the current study.

The effects of pressure treatments on myofibrillar protein solubility are not fully understood. Studies on the high hydrostatic pressure treatment of meat homogenates and extracted myofibrils have shown that high pressure increases myofibrillar protein solubility (Macfarlane 1974; Macfarlane and McKenzie 1976). Using bovine *biceps femoris* muscles, however, Schilling *et al*. (2002) observed that HDP had no effect on myofibrillar or sarcoplasmic protein solubility. Variations are likely a result of differences in the magnitude of pressure and duration of treatment used in these studies. At first glance, data from the current study support that of Schilling *et al*. (2002). Closer examination, however, reveals that except for two of the samples, the myofibrillar protein solubility was 5 to 30% higher in HDP- and BT + HDP-treated samples compared with nontreated controls. When accounting for these outliers, the data suggest that HDP shock waves may enhance myofibrillar protein solubility. Further research is warranted, however. Besides demonstrating a direct effect of high-pressure shock waves on muscle proteins, increased myofibrillar protein solubility with HDP treatment also indicates that HDP may have beneficial effects on protein functionality and further processed meat characteristics.

Using SDS-PAGE analysis, the compositions of the myofibrillar and sarcoplasmic protein fractions were determined in order to investigate the potential role of protein degradation in the tenderizing effects of the treatments. Because BT is based on the mechanical severance of the muscle tissue, as expected no differences in the protein profiles were observed between BT-treated and paired control samples (Figs. 1 and 2). The composition of the sarcoplasmic protein profile varied among top rounds but was relatively unaffected by any of the treatments. Some data suggest that changes in sarcoplasmic protein composition may serve as indicators of tenderness. Using capillary electrophoresis, Patel *et al*. (2006) observed that changes in the content of 30- and 100-kDa proteins in the sarcoplasmic fraction from beef strip loins were correlated to shear force values at 2 days postmortem. Similarly, the current study found that the abundance of 90- and 29-kDa sarcoplasmic proteins was significantly correlated to shear force measurements. However, there was not a strong relationship between tenderness and the overall composition of the sarcoplasmic protein fraction in the current study.

Minor differences in the myofibrillar protein profiles existed between HDP and BT + HDP samples and their respective controls. No treatment differences were detected in myosin or actin bands, the predominant proteins of the contractile apparatus. Differences did exist, however, in the ~100 to 110-kDa band densities in samples receiving HDP treatment. Compared with controls, HDP and BT + HDP treatments caused an increase in the density of this band, typically identified as α-actinin. This suggests that either HDP increases the release and extractability of α-actinin from the Z-line, or a 100 to 110-kDa protein fragment is generated from the degradation of a higher molecular weight protein due to HDP. The latter explanation is supported by previous studies that observed the appearance of a 110-kDa protein fragment in the myofibrillar protein fraction during the aging tenderization of beef (Koohmaraie *et al*. 1984; O'Halloran *et al*. 1997). Sequence analysis of the 110-kDa myofibrillar protein fragment has shown strong homology with C-protein (Casserly *et al*. 1998). Because C-protein helps to stabilize thick filaments, it is reasonable to postulate that the degradation of this protein may contribute to the tenderizing effect of HDP. Additional studies are required to further identify and characterize HDP-induced changes in the myofibrillar protein profile.

Overall, differences in the myofibrillar protein composition were minimal and not highly correlated to day 0 or day 7 tenderness measurements. One possibility for the lack of myofibrillar protein differences and synergistic treatment effects may have been that the top rounds were aged 7 days post-mortem prior to freezing. Past research has demonstrated that the degradation of myofibrillar and cytoskeletal proteins such as titin, nebulin, filamin, desmin and troponin-T are closely associated with aging-related changes in meat tenderness (Taylor *et al*. 1995; Huff-Lonergan *et al*. 1996). Any HDP-induced protein degradation that influences tenderness is likely to occur in these proteins. The relatively low abundance of intact desmin and the high content of the 30-kDa band (breakdown product of troponin-T) in control samples indicate degradation of tenderness-related proteins prior to treatment. Thus, tenderness-related protein degradation because of treatments would probably be minimized. Overall, the lack of differences observed in the myofibrillar proteins and the low correlation of collagen changes with tenderness suggest that the majority of the HDP-tenderizing effect is manifested through the physical disruption of the myofibrils.

CONCLUSIONS

BT and HDP treatments are both effective methodologies for tenderizing top rounds from Brahman cattle. The results from this study suggest that the

mechanism of tenderization is different between these techniques. Overall, no synergistic effects were observed for any of the measured parameters with the combination BT + HDP treatment. The fact that BT samples had an increased MFI and no protein solubility or SDS-PAGE banding pattern differences compared with controls, confirms that BT likely tenderizes muscle by physically disrupting and severing the connective tissue and myofibrillar structure. Shifts in the SDS-PAGE banding patterns of myofibrillar proteins and increased MFI and collagen solubility with HDP and BT + HDP treatments indicate that in addition to the physical disruption to the muscle structure, HDP treatment may influence meat tenderness through direct alterations of myofibrillar proteins and collagen solubility.

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