EFFECT OF HYDRODYNAMIC PRESSURE PROCESSING AND AGING ON THE TENDERNESS AND MYOFIBRILLAR PROTEINS OF BEEF STRIP LOINS*

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ABSTRACT

*This study evaluated the effects of hydrodynamic pressure (HDP) processing and aging on the tenderness and myofibrillar proteins of beef strip loins. Loins (*n = *12) were halved at 48 h postmortem and assigned to HDP or control treatments. Following treatment, each half was divided into three portions for aging (0, 5 or 8 days). Samples were removed for Warner–Bratzler shear force (WBSF) determination and myofibrillar protein isolation. HDP decreased (*P < *0.0001) WBSF values 23% at 0, 5 and 8 days of aging. Myofibrillar fragmentation and myofibrillar protein solubility increased (*P < *0.01) with HDP and aging. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blotting analysis of myofibrillar proteins showed that HDP and aging decreased the intensity of the troponin T (TnT) band and enhanced the accumulation of the 30 kD TnT degradation product. These data suggest that HDP is more effective than aging tenderization, and that HDP tenderization is caused by both protein degradation and physical disruption of the myofibril apparatus.*

PRACTICAL APPLICATIONS

Hydrodynamic pressure (HDP) processing was shown to instantaneously tenderize tough cuts of beef to tenderness levels attained following 8 days of

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postmortem aging. For the meat industry, these results demonstrate the potential benefits of alternative tenderization techniques, such as high-energy shock waves. Observed changes in protein characteristics due to HDP and aging allow researchers to understand better potential mechanisms of meat tenderization.

INTRODUCTION

Meat quality is determined by a number of attributes, including texture, flavor, color and juiciness. Tenderness is consistently ranked by consumers as the primary quality characteristic that determines eating satisfaction (Huffman *et al.* 1996; Miller *et al.* 2001). Persistent tenderness problems within the beef industry have led to much research into postharvest tenderization techniques. Because meat tenderness is strongly influenced by myofibrillar protein interactions and muscle ultrastructure (Locker 1960; Marsh and Leet 1966), postharvest techniques to improve tenderness often influence the myofibrillar component of the muscle. Aging tenderization is strongly associated with the proteolytic breakdown of key myofibrillar and cytoskeletal proteins such as titin, nebulin, filamin, desmin and troponin T (TnT) (Taylor *et al.* 1995; Huff-Lonergan *et al.* 1996). Unfortunately, aging product until it reaches an acceptable level of tenderness costs the industry time and money. The development of new technologies to more rapidly tenderize meat or enhance the aging process would be of great benefit to the meat industry.

Hydrodynamic pressure (HDP) processing is a novel postharvest tenderization technique in which high-energy shock waves in water cause instantaneous tenderization of meat (Solomon *et al.* 1997). Although HDP has repeatedly been shown to tenderize a variety of meat products (Solomon *et al.* 1998; Moeller *et al.* 1999; Claus *et al.* 2001; Marriott *et al.* 2001), the underlying mechanism of tenderization is not well understood. Furthermore, there is a lack of data elucidating the tenderization effect of combining HDP and aging treatments, and their influence on muscle proteins related to meat quality. Thus, the objective of this study was to determine the influence of HDP and aging on the tenderness and myofibrillar protein characteristics of fresh, boneless beef strip loins.

MATERIALS AND METHODS

Muscle Samples

Twenty-four boneless strip loins were obtained from a commercial beef-processing facility from Select, Yield Grade 2 and 3 beef carcasses at 24 h

postmortem. After the removal of the sirloin portion, one 2.5-cm-thick steak was removed from the anterior end of each strip loin for baseline determination of Warner–Bratzler shear force (WBSF). The 12 strip loins selected for use in this experiment had shear force values ranging from 6.77 to 12.84 kgf at 24 h postmortem.

Experimental Design

Strip loins were processed on four separate days with three strip loins per replication. At 48 h postmortem (designated as experimental day 0), each strip loin was split into anterior and posterior halves, which were assigned either control or HDP treatments such that each loin served as its own control. Following treatments (HDP and control) at day 0, each half strip loin was split into thirds which were assigned to either 0, 5 or 8 days of aging. All samples were then vacuum packaged and stored at 4C for the designated aging period. Following aging, one 2.5-cm-thick steak was removed from each section for WBSF determination, and a second 2.5-cm-thick steak was removed for protein analysis. To account for inherent variation in tenderness along the length of the strip loins, a completely balanced design was used such that each treatment–aging combination was applied to each anatomical position along the length of the strip loins an equal number of times. Steaks used for protein analysis were trimmed free of fat, knife minced and thoroughly mixed prior to sampling in order to account for the inherent variation within a steak.

HDP Treatments

The parameters of the HDP treatment were set according to the findings of past studies described in Solomon *et al.* (2006). Strip loin sections ($15 \times 17.5 \times 6.3$ cm; 2.0–2.2 kg) designated for HDP treatment were individually packaged in boneguard bags (Cryovac/Sealed Air Corp., Duncan, SC), briefly heat shrunk (~87C) and placed onto a 1.3-cm-thick flat metal disk inside a 98 L plastic container filled with water (4–6C). The container was suspended 25 cm above the floor, and a 100 g rectangular binary explosive was detonated 31 cm above the meat to generate the high-energy shock waves. One sample was treated per HDP process.

Tenderness Evaluation

Steaks (2.5-cm thick) were cooked from an initial temperature of 4–6C to an end point temperature of 71C on an electric grill (model GGR50B, Salton, Inc., Mt. Prospect, IL) according to AMSA (1995) guidelines. Temperature was monitored in the geometric center of each steak with a type J thermocouple wire connected to a temperature meter (model HH21, Omega Engi-

neering, Stanford, CT). Steaks were turned once when the internal temperature reached 40C. Steaks were cooled to room temperature, and a coring device with an inner diameter of 1.27 cm was utilized to remove cores parallel to muscle fiber direction. WBSF values (kgf) were measured by shearing cores (11–20 per steak) perpendicular to muscle fibers with a meat shear blade (1.8-mm thick) mounted to a texture analyzer (model TMS-90, Food Technology Corp., Sterling, VA).

Myofibrillar Fragmentation Index (MFI)

MFI was determined on duplicate 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 [EDTA], 1 mM NaN_3, 25 mM potassium phosphate [pH 7.0]) 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 openings) 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 1,000 × g (2C), and the supernatant was decanted. The myofibril pellet was suspended in 10 mL of buffer by a vortex mixer and centrifuged for 10 min at 1,000 × 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, MD), and the mean of the triplicate absorbance readings was multiplied by 150 to give the index value for MFI.

Protein Solubility

Protein solubility was measured using the procedure of Schilling *et al.* (2002) with modifications. Total protein solubility was determined by homogenizing duplicate 1-g muscle samples with 10 mL of ice-cold 1.0 M KI/0.1 M potassium phosphate (pH 7.2) buffer in four 4-s bursts with a Kinematica polytron (model PT 10/35, Brinkman Instruments, Inc., Westbury, NY). The homogenate was incubated overnight at 4C and then centrifuged at 2,600 × g for 30 min. The supernatant was decanted and protein concentration was measured using the biuret method. Sarcoplasmic protein solubility was similarly determined in 0.025 M potassium phosphate (pH 7.2) buffer. Myofibrillar

protein solubility was calculated from the difference between the total and sarcoplasmic protein solubility. Values are expressed as milligrams of protein per gram of muscle tissue.

Myofibrillar Protein Isolation

The myofibrillar protein fraction was isolated according to the procedure of Goll *et al.* (1974) with modifications. Trimmed muscle samples were first minced for 5 s in a handheld blender (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 ethylene glycol tetraacetic acid, 1 mM NaN_3) using a Waring blender (Waring, Winsted, CT) equipped with a rheostat (Powerstat, Superior Electric Co., Bristol, CT) at a setting of 65% for 10 s. The homogenate was filtered through two layers of cheesecloth and centrifuged at 1,500 × g for 20 min (4C). The resulting pellet was resuspended in 20 mL of SSS, homogenized using three 4-s bursts with a Kinematica polytron, and centrifuged at 1,500 × g for 10 min. The pellet was then resuspended, homogenized and centrifuged twice more using SSS. The pellet was resuspended and washed two times in SSS + 1% Triton X-100 (Bio-Rad Laboratories, Hercules, CA), and centrifuged (1,500 × g for 10 min). The pellet was then resuspended and washed two additional times in 15 mL of SSS and centrifuged at 1,500 × 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(hydroxymethyl)aminomethane [Tris] [pH 7.0]) and 50% (v/v) glycerol.

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

Protein concentrations of isolated myofibril samples were determined using the biuret method and adjusted to 2 mg/mL. 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 [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. For evaluation of the myofibrillar protein profile, denatured myofibril samples were loaded (10 µg per lane) onto precast 4–20% acrylamide gradient Tris–HCl gels. Gels were run in duplicate on the Bio-Rad Criterion gel system (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, 2 mM EDTA and 0.1% (w/v) SDS. Gels were stained in a solution of 0.05% Coomassie brilliant blue R-250, 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. For better visualization of lower-molecular-weight proteins and

Western blotting, myofibril samples were also run on precast 15% acrylamide Tris–HCl gels. Gels transferred for Western blotting were not stained. Stained 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 density of protein bands. Broad range (6.5–200 kDa) molecular weight standards (Bio-Rad Laboratories) were run on each gel to determine the protein band molecular weights and to account for gel-to-gel variations. To account for slight variations in protein loading, the density of each protein band was expressed as a percentage of the total density of all the protein bands within the lane. Because of potential protein-to-protein variation in Coomassie staining, comparisons were only made within a given protein band across samples.

Transfer Conditions and Western Blotting

Prior to transfer, gels were equilibrated for 15 min in cold transfer buffer (25 mM Tris, 192 mM glycine, 2 mM EDTA, 15% [v/v] methanol). The samples were transferred onto PVDF membranes using a Criterion Blotter unit (Bio-Rad Laboratories) at a constant voltage setting of 90 V for 15 min. The temperature of the transfer buffer was kept at approximately 4C during the transfer by a circulating cooling unit (model RTE-110, NESLAB Instruments, Inc., Newington, NH). Following transfer, membranes were incubated for 1 h at 25C in a blocking solution (10 mM phosphate-buffered saline [PBS] [pH 7.4], 0.1% [v/v] Tween-20 and 3% [w/v] nonfat dry milk). Following the manufacturer's protocols, Western blots were conducted using a colorimetric horseradish peroxidase detection system (Amplified Opti-4CN Detection Kit, Bio-Rad Laboratories). Blots were incubated in primary antibody, monoclonal anti-TnT (JLT-12, Sigma Chemical, St. Louis, MO) diluted 1:30,000 in PBS–Tween, for 1.5 h at 25C and washed three times (10 min per wash) with PBS–Tween. Blots were then incubated in goat anti-mouse IgG horseradish peroxidase-conjugated secondary antibody (Bio-Rad Laboratories) diluted 1:25,000 in PBS–Tween for 1 h at 25C. Blots were washed three times (10 min per wash) with PBS–Tween prior to amplification and detection steps.

pH

From the steaks removed for protein analysis, pH was measured according to the procedure of Solomon (1987) on homogenized 2-g muscle samples in duplicate using a pH electrode attached to a digital pH meter (model 330, Orion Research, Inc., Boston, MA).

Statistical Analysis

Data were analyzed as a two-way analysis of variance using the PROC MIXED procedure of SAS (version 9.1, 2002–2003, SAS Institute, Inc., Cary,

NC). For the analysis of all parameters except WBSF, the model included treatment (control or HDP), aging (0, 5 or 8 days) and the treatment by aging interaction as fixed effects and strip loin as a random block effect. The model for WBSF measurements utilized similar fixed effects, but included 24 h postmortem WBSF measurements used for sample prescreening as a covariate instead of strip loin as a random effect. The anatomical position of each treatment–aging combination within each strip loin and the half (anterior or posterior) that was assigned HDP treatment was analyzed and found to have nonsignificant effects on the dependent variables measured. Thus, the experimental design adequately accounted for inherent variations along the length of the strip loins, and these terms were not included in the final model. Significant differences ($P < 0.05$) between means were separated with the PDIFF option. Correlation coefficients were determined using the PROC CORR procedure.

RESULTS

Effects of HDP and Aging on Tenderness

HDP and aging improved the tenderness of beef strip loins; however, there was not a significant treatment by aging interaction effect on WBSF (Fig. 1). Following all three aging periods, HDP loins had lower ($P < 0.0001$) WBSF measurements than the controls. The improvement in tenderness with HDP treatment compared to the controls was approximately 23% at 0, 5 and 8 days of aging. There was a strong aging effect ($P < 0.0001$) from days 0 to 5 as WBSF decreased 20%, and another lesser effect of 11% from days 5 to 8 in

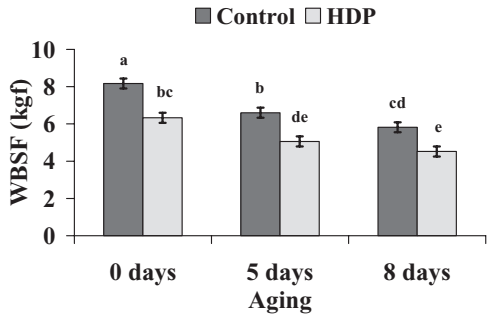

FIG. 1. LEAST SQUARE MEANS AND STANDARD ERRORS OF THE WARNER–BRATZLER SHEAR FORCE (WBSF) VALUES OF CONTROL AND HYDRODYNAMIC PRESSURE (HDP) BEEF STRIP LOINS TREATED AT 48 H POSTMORTEM AND THEN AGED 0, 5 AND 8 DAYS
Bars with different superscripts are significantly different ($P < 0.05$).

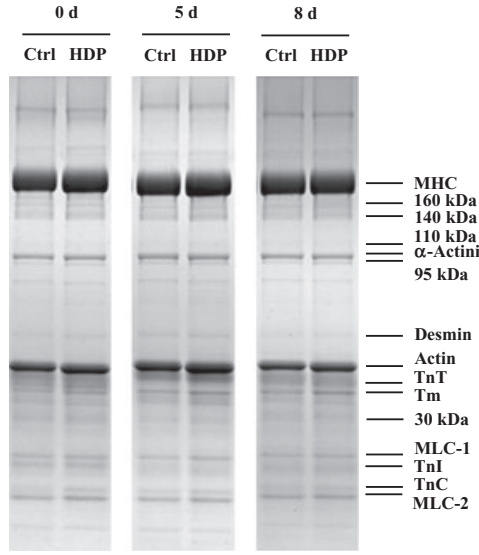

FIG. 2. SDS-PAGE (4–20% GRADIENT GELS) OF MYOFIBRILS FROM CONTROL AND HYDRODYNAMIC PRESSURE (HDP) BEEF STRIP LOINS TREATED AT 48 H POSTMORTEM AND THEN AGED 0, 5 AND 8 DAYS
MHC, myosin heavy chain; TnT, troponin T; Tm, tropomyosin; MLC-1, myosin light chain-1; TnI, troponin I; TnC, troponin C; MLC-2, myosin light chain-2.

both treated and control samples. Day 0 HDP samples exhibited equivalent WBSF measurements to day 8 control samples. Compared to day 0 control samples, aging for 8 days improved the tenderness 29% whereas aging for 8 days following HDP treatment improved the tenderness 45%. When calculated from WBSF values measured at 24 h postmortem (data not shown), the rate of tenderness improvement from 24 h to 10 days postmortem was greater in HDP loins (–0.47 kgf/day) compared to control loins (–0.33 kgf/day).

Effects of HDP and Aging on MFI, Protein Solubility and pH

The effects of HDP and aging on the MFI, protein solubility and pH measurements of beef strip loins are shown in Table 1. The MFI values were strongly influenced by HDP and aging, but there was not a significant treatment by aging interaction effect. MFI was 25% higher ($P < 0.001$) in HDP versus control samples at day 0. After 5 and 8 days of aging, the difference between HDP and control samples was not significant ($P > 0.05$). From 0 to 5 days of aging, the MFI increased ($P < 0.0001$) approximately 36% in the HDP samples and 48% in the control samples. The MFI values were not statistically

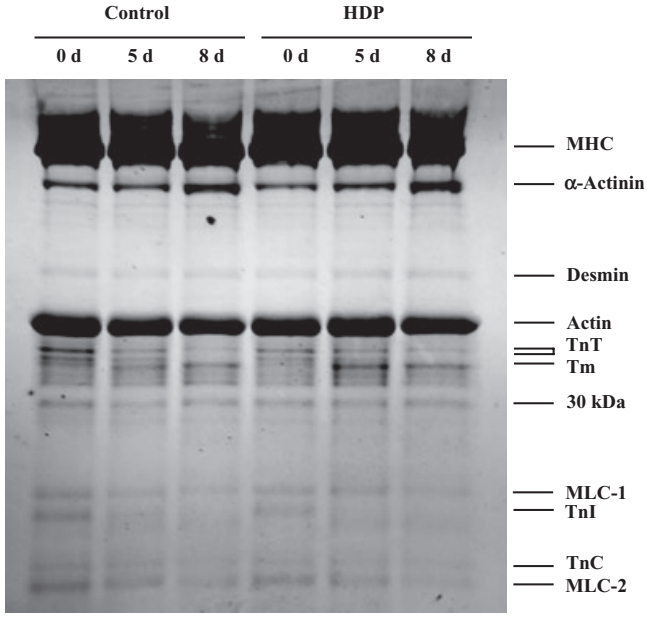

FIG. 3. SDS-PAGE (15% GELS) OF MYOFIBRILS FROM CONTROL AND HYDRODYNAMIC PRESSURE (HDP) BEEF STRIP LOINS TREATED AT 48 H POSTMORTEM AND THEN AGED 0, 5 AND 8 DAYS
MHC, myosin heavy chain; TnT, troponin T; Tm, tropomyosin; MLC-1, myosin light chain-1; TnI, troponin I; TnC, troponin C; MLC-2, myosin light chain-2.

different between 5 and 8 days of aging in either HDP or control samples. Similarly, myofibrillar protein solubility data showed significant HDP and aging effects ($P < 0.0001$ and $P < 0.05$, respectively), but no treatment by aging interaction effect. At all three aging periods, the HDP samples had higher ($P < 0.0001$) myofibrillar protein solubility than the controls. In both HDP and control samples, myofibrillar protein solubility did not change from 0 to 5 days, but increased ($P < 0.05$) from 5 to 8 days of aging. Total protein solubility was higher ($P < 0.01$) in HDP samples compared to controls at 0 and 8 days of aging. Overall, there were no significant aging or treatment by aging interaction effects on total protein solubility. While there was loin-to-loin variation in muscle pH, there were no significant HDP or aging effects on pH in this experiment.

Effects of HDP and Aging on SDS-PAGE Myofibrillar Protein Profile

The effects of HDP and aging treatments on the electrophoretic profile of myofibrils isolated from beef strip loins are shown in Table 2. A representative

TABLE 1.
MYOFIBRILLAR FRAGMENTATION INDEX (MFI), PROTEIN SOLUBILITY AND pH VALUES OF CONTROL AND HYDRODYNAMIC PRESSURE (HDP) BEEF STRIP LOINS TREATED AT 48 H POSTMORTEM AND THEN AGED 0, 5 AND 8 DAYS

Parameter	Treatment	Aging period (days)			SEM	*P* value		
		0	5	8		Treatment	Aging	Treatment × aging
MFI	Control	69.2^{d}	102.5^{bc}	116.7^{ab}	7.8	<0.01	<0.0001	NS
	HDP	86.7^{c}	117.8^{ab}	128.0^{a}				
MPS	Control	125.9^{c}	127.3^{c}	135.7^{b}	3.4	<0.0001	<0.05	NS
	HDP	133.6^{b}	134.4^{b}	144.8^{a}				
TPS	Control	193.0^{c}	191.8^{c}	196.2^{bc}	3.3	<0.01	NS	NS
	HDP	200.3^{ab}	196.0^{bc}	205.2^{a}				
pH	Control	5.44	5.48	5.49	0.02	NS	NS	NS
	HDP	5.50	5.48	5.51				

NS indicates not significant ($P > 0.05$).
[a–d] Least square means across all aging periods and treatments with different superscripts differ significantly ($P < 0.05$).
SEM, standard error of means; MFI, myofibrillar fragmentation index; MPS, myofibrillar protein solubility (mg protein/g muscle tissue); TPS, total protein solubility (mg protein/g muscle tissue).

image of the gels used for quantification of individual protein bands is depicted in Fig. 2. None of the protein bands showed a significant HDP by aging interaction effect. Bands corresponding to myosin heavy chain (MHC), actin, myosin light chain-1 (MLC-1), troponin C (TnC) and myosin light chain-2 (MLC-2) were not influenced by HDP or aging treatments ($P > 0.05$). The two bands immediately below MHC, 160 and 140 kDa, respectively, each increased ($P < 0.01$) in intensity with aging from 0 to 5 and 8 days, but were not affected by HDP treatment. Similarly, the α-actinin band was not influenced by HDP, but the intensity of the α-actinin band increased ($P < 0.0001$) with aging from 0 to 5 and 8 days. The desmin band showed a slight decrease ($P < 0.0001$) in intensity from 0 to 5 and 8 days in both HDP and control samples. The TnT band which appears as a doublet was influenced by both HDP ($P < 0.05$) and aging ($P < 0.05$). Both HDP and control samples showed a decrease in TnT between 0 and 5 days with no significant change between 5 and 8 days. Overall, the HDP samples had lower TnT band intensities than the controls. Tropomyosin (Tm) bands showed a slight increase ($P < 0.0001$) in intensity from 0 to 5 and 8 days in both HDP and control samples with no significant HDP effect. The 30 kDa protein was influenced by HDP and aging treatments. In both HDP and control samples, the intensity of the 30 kDa band increased ($P < 0.01$) with aging. The intensity of the 30 kDa band was greater ($P < 0.001$) in HDP versus control samples across all aging periods. Troponin

TABLE 2.
CONTENT* OF MYOFIBRILLAR PROTEINS FROM CONTROL AND HYDRODYNAMIC PRESSURE (HDP) BEEF STRIP LOINS TREATED AT 48 H POSTMORTEM AND THEN AGED 0, 5 AND 8 DAYS AS DETERMINED BY SDS-PAGE ANALYSIS

Protein band	Treatment	Aging period (days)			Mean‡	*P* value		
		0	5	8		Treatment	Aging	Treatment × aging
MHC	Control	32.28	31.12	32.46	31.95	NS	NS	NS
	HDP	31.12	30.68	31.45	31.08			
	Mean†	31.70	30.90	31.96				
160 kDa	Control	0.63	1.01	1.22	0.96	NS	<0.001	NS
	HDP	0.50	1.24	1.21	0.98			
	Mean†	0.56[b]	1.13[a]	1.22[a]				
140 kDa	Control	1.08	1.62	1.64	1.45	NS	<0.01	NS
	HDP	0.85	1.98	1.66	1.50			
	Mean†	0.96[b]	1.80[a]	1.65[a]				
α-Actinin	Control	2.88	3.49	3.55	3.31	NS	<0.0001	NS
	HDP	2.68	3.39	3.49	3.19			
	Mean†	2.78[b]	3.44[a]	3.52[a]				
Desmin	Control	1.50	1.42	1.24	1.39	NS	<0.0001	NS
	HDP	1.52	1.29	1.05	1.29			
	Mean†	1.51[a]	1.36[b]	1.15[b]				
Actin	Control	15.05	15.62	14.95	15.21	NS	NS	NS
	HDP	15.83	15.58	14.36	15.26			
	Mean†	15.44	15.60	14.66				
TnT	Control	6.43	5.70	5.81	5.98[a]	<0.05	<0.05	NS
	HDP	6.31	4.97	5.51	5.60[b]			
	Mean†	6.37[a]	5.34[b]	5.66[b]				
Tm	Control	3.77	4.86	4.82	4.48	NS	<0.0001	NS
	HDP	4.24	4.71	5.30	4.75			
	Mean†	4.01[b]	4.78[a]	5.06[a]				
30 kDa	Control	3.06	4.11	4.20	3.79[b]	<0.001	<0.01	NS
	HDP	3.87	4.49	5.13	4.50[a]			
	Mean†	3.47[b]	4.30[a]	4.67[a]				
MLC-1	Control	3.11	3.24	2.71	3.02	NS	NS	NS
	HDP	2.90	3.07	2.97	2.98			
	Mean†	3.01	3.15	2.84				
TnI	Control	2.87	1.92	1.50	2.10	NS	<0.0001	NS
	HDP	2.86	1.94	1.65	2.15			
	Mean†	2.87[a]	1.93[b]	1.58[b]				
TnC	Control	2.01	1.93	1.99	1.98	NS	NS	NS
	HDP	1.85	1.98	1.78	1.87			
	Mean†	1.93	1.95	1.88				
MLC-2	Control	3.68	3.21	3.26	3.38	NS	NS	NS
	HDP	3.64	3.32	3.24	3.40			
	Mean†	3.66	3.27	3.25				

NS indicates not significant ($P > 0.05$).
* Values represent the least square means of the intensity of each SDS-PAGE band expressed as a percentage of the total intensity of all bands in the sample.
† Mean intensity of protein bands within each aging period.
‡ Mean intensity of protein bands within each treatment.
[ab] Mean intensities with different superscripts differ significantly ($P < 0.05$).
MHC, myosin heavy chain; TnT, troponin T; Tm, tropomyosin; MLC-1, myosin light chain-1; TnI, troponin I; TnC, troponin C; MLC-2, myosin light chain-2.

TABLE 3.
CORRELATIONS BETWEEN TENDERNESS, MYOFIBRILLAR FRAGMENTATION INDEX (MFI), PROTEIN SOLUBILITY, pH AND SDS-PAGE BAND INTENSITIES OF MYOFIBRILLAR PROTEINS FROM BEEF STRIP LOINS ACROSS ALL TREATMENTS AND AGING PERIODS

Protein bands	WBSF	MFI	MPS	TPS	pH
MFI	–0.75***				
MPS	–0.45***	0.40***			
TPS	–0.22	0.22	0.83***		
pH	0.07	–0.09	0.13	0.03	
MHC	0.11	–0.10	–0.24*	–0.10	0.03
160 kDa	–0.23*	0.21	0.19	0.10	–0.05
140 kDa	–0.17	0.06	0.12	0.07	–0.04
α-Actinin	–0.38**	0.40***	0.23	0.13	0.17
Desmin	0.23*	–0.25*	–0.13	–0.02	0.18
Actin	0.12	–0.16	–0.23*	–0.03	0.04
TnT	0.23*	–0.33**	–0.22*	–0.15	0.15
Tm	–0.47***	0.51***	0.37**	0.10	0.10
30 kDa	–0.56***	0.50***	0.26*	–0.06	0.09
MLC-1	–0.20	0.22	–0.01	–0.07	–0.06
TnI	0.36**	–0.39***	–0.25*	0.02	–0.25
TnC	–0.12	0.22	0.14	0.03	–0.23
MLC-2	–0.11	0.17	0.09	0.15	–0.18

Levels of significance: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.
WBSF, Warner–Bratzler shear force; MFI, myofibrillar fragmentation index; MPS, myofibrillar protein solubility; TPS, total protein solubility; MHC, myosin heavy chain; TnT, troponin T; Tm, tropomyosin; MLC-1, myosin light chain-1; TnI, troponin I; TnC, troponin C; MLC-2, myosin light chain-2.

I (TnI) band intensity decreased ($P < 0.0001$) from 0 to 5 and 8 days of aging in both HDP and control samples, but there was not a significant HDP effect.

Relationships Between WBSF, MFI, Protein Solubility, pH, and SDS-PAGE Myofibrillar Protein Profile

Table 3 shows the correlations between WBSF, MFI, protein solubility, pH and the SDS-PAGE myofibrillar protein profiles of beef strip loins across both treatments and aging periods. As expected, there was a high correlation between WBSF and MFI values ($r = –0.75$). Changes in myofibrillar protein solubility were associated with WBSF and MFI values ($r = –0.45$ and 0.40, respectively), but total protein solubility and pH measurements were not. Evaluation of SDS-PAGE band intensity data shows that WBSF and MFI were most strongly correlated to the abundance of the 30-kDa band. Weaker associations were found between WBSF and MFI measurements and the intensity of bands corresponding to 160 kDa, α-actinin, desmin, TnT, Tm and TnI. WBSF and MFI values were not correlated to the intensity of bands

TABLE 4.
CORRELATIONS BETWEEN TENDERNESS MEASUREMENTS AT 0, 5 AND 8 DAYS AND MYOFIBRILLAR FRAGMENTATION INDEX (MFI), PROTEIN SOLUBILITY, pH AND SDS-PAGE BAND INTENSITIES OF MYOFIBRILLAR PROTEINS FROM BEEF STRIP LOINS

	Aging (days)	WBSF		
		0 days	5 days	8 days
MHC	0	0.31	0.05	–0.01
160 kDa	0	–0.14	0.10	–0.08
140 kDa	0	0.06	0.28	0.07
α-Actinin	0	0.20	0.16	–0.06
Desmin	0	0.31	0.27	0.17
Actin	0	–0.01	0.06	0.12
TnT	0	–0.15	–0.12	0.02
Tm	0	–0.07	–0.25	–0.24
30 kDa	0	–0.52*	–0.56**	–0.38*
MLC-1	0	0.05	–0.09	–0.01
TnI	0	–0.19	–0.20	–0.27
TnC	0	–0.08	–0.04	0.01
MLC-2	0	–0.32	–0.25	–0.29
WBSF	0	1.00	0.87***	0.81***
	5	0.87***	1.00	0.86***
	8	0.81***	0.86***	1.00
MFI	0	–0.71***	–0.71***	–0.60**
	5	–0.56**	–0.60**	–0.62***
	8	–0.52**	–0.71***	–0.72***
MPS	0	–0.30	–0.03	–0.10
	5	–0.39*	–0.30	–0.25
	8	–0.56**	–0.58**	–0.55**
TPS	0	–0.22	–0.01	–0.06
	5	–0.11	–0.02	0.15
	8	–0.32	–0.41*	–0.36*
pH	0	0.13	0.10	0.06
	5	0.29	0.17	0.11
	8	0.24	0.35*	0.46*

Levels of significance: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.
WBSF, Warner–Bratzler shear force; MFI, myofibrillar fragmentation index; MPS, myofibrillar protein solubility; TPS, total protein solubility; MHC, myosin heavy chain; TnT, troponin T; Tm, tropomyosin; MLC-1, myosin light chain-1; TnI, troponin I; TnC, troponin C; MLC-2, myosin light chain-2.

corresponding to MHC, 140 kDa, actin, MLC-1, TnC and MLC-2. Myofibrillar protein solubility was correlated to MHC, actin, TnT, Tm, 30-kDa and TnI bands, but the correlation was low ($r = –0.25$ to 0.37). Neither total protein solubility nor pH was correlated to WBSF, MFI or SDS-PAGE band intensities.

Table 4 shows the correlation between WBSF measurements after different aging periods and the other parameters measured in this study. As

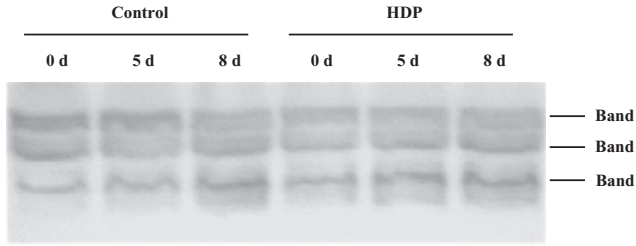

FIG. 4. WESTERN BLOT OF MYOFIBRILS ISOLATED FROM CONTROL AND HYDRODYNAMIC PRESSURE (HDP) BEEF STRIP LOINS TREATED AT 48 H POSTMORTEM AND THEN AGED 0, 5 AND 8 DAYS
Blot prepared from 15% gel and labeled with monoclonal troponin T antibody.

expected, there was a strong correlation between the MFI values and the WBSF at 0, 5 and 8 days of aging ($r = -0.52$ to -0.72). Overall, the level of myofibrillar protein solubility at 0 and 5 days was not significantly correlated to WBSF measurements. Myofibrillar protein solubility measurements at 8 days, however, were correlated to WBSF. Except when measured at 8 days, total protein solubility and pH measurements were not correlated with WBSF. From the SDS-PAGE myofibrillar protein profiles at day 0, only the 30-kDa band was significantly correlated to WBSF. The intensity of the 30-kDa band at day 0 was correlated to WBSF measurements after 0, 5 and 8 days of aging ($r = -0.38$ to -0.56).

Effects of HDP and Aging on TnT Degradation

To verify the effects of HDP and aging on TnT degradation, the isolated myofibrils were separated on a 15% gel (Fig. 3), transferred to PVDF membrane and analyzed by a Western blot with TnT-specific antibody (Fig. 4). The Western blot identified bands corresponding to intact TnT (band 1), an intermediate TnT fragment (band 2) and a 30-kDa TnT fragment (band 3) in both control and HDP samples (Fig. 4). The abundance of each band was expressed as the ratio between the intensity of the band of interest and the total intensity of all bands within the lane (Table 5). With aging, the intensity of band 1 decreased ($P < 0.01$) and band 3 increased ($P < 0.01$). The intensity of band 1 decreased and the intensity of band 3 increased with HDP treatment compared to the controls. The relative intensity of band 2 was not significantly affected by HDP or aging treatments. While there was not a statistically significant treatment by aging interaction effect for any of the bands, individual comparison of treatment by aging combinations indicated that HDP samples had less intact TnT (band 1) and more of the 30-kDa TnT fragment (band 3) at 0 and 5 days of aging compared to controls.

TABLE 5.
RELATIVE ABUNDANCE* OF TROPONIN T (TnT) IN WESTERN BLOTS OF MYOFIBRILS FROM CONTROL AND HYDRODYNAMIC PRESSURE (HDP) BEEF STRIP LOINS TREATED AT 48 H POSTMORTEM AND THEN AGED 0, 5 AND 8 DAYS

(TnT) band	Treatment	Aging period (days)			Mean‡	*P* value		
		0	5	8		Treatment	Aging	Treatment × aging
Band 1	Control	0.515	0.389	0.297	0.401[a]	<0.01	<0.01	NS
	HDP	0.348	0.274	0.285	0.305[b]			
	Mean†	0.432[a]	0.332[b]	0.291[b]				
Band 2	Control	0.275	0.266	0.294	0.277	NS	NS	NS
	HDP	0.301	0.260	0.289	0.279			
	Mean†	0.288	0.263	0.292				
Band 3	Control	0.210	0.345	0.409	0.322[b]	<0.05	<0.01	NS
	HDP	0.351	0.466	0.427	0.416[a]			
	Mean†	0.281[b]	0.406[a]	0.418[a]				

NS indicates not significant ($P > 0.05$).
* Values represent the least square means of the band intensities expressed as a proportion of the total intensity of all bands in the sample.
† Mean intensity of bands within each aging period.
‡ Mean intensity of bands within each treatment.
[ab] Mean intensities with different superscripts differ significantly ($P < 0.05$).

DISCUSSION

The objective of the current study was to determine the effects of HDP and aging on tenderness and related myofibrillar protein changes in beef strip loins in order to understand better mechanisms of meat tenderization. Past research has found that high-energy shock waves can instantaneously improve meat tenderness in a variety of meat products (Solomon *et al.* 1998; Moeller *et al.* 1999; Claus *et al.* 2001; Marriott *et al.* 2001; Liu *et al.* 2006) with virtually no effect on other sensory properties (Berry *et al.* 1997). Additionally, HDP-treated beef has been previously shown to age faster and to a greater extent than nontreated controls (Solomon *et al.* 2002). Consistent with these findings, HDP-treated samples in the current study not only demonstrated instantaneous tenderness improvements (23% improvement at day 0), but also achieved a greater overall level of tenderness than control samples with aging for 8 days (4.52 versus 5.82 kgf). Control samples required 8 days of aging to attain the level of tenderness achieved by day 0 HDP samples. These data demonstrate the effectiveness of using high-energy shock waves to tenderize post-rigor muscle tissue.

Meat tenderization often occurs because of ultrastructural changes that weaken the integrity of the myofibers in the muscle tissue. Aging-related

improvements in meat tenderness have been associated with intra-myofibrillar breaks at the junction of the I-band and Z-line, loss of Z-line attachments between adjacent myofibrils and increased inter-myofibrillar spacing (Davey and Dickson 1970; Taylor *et al.* 1995; Ho *et al.* 1996). Similarly, the instantaneous tenderization effect of HDP is thought to be caused by the physical disruption that occurs within and between myofibrils. Using transmission electron microscopy, Zuckerman and Solomon (1998) demonstrated that high-energy shock waves cause myofibrillar fragmentation in the I-band region adjacent to the Z-lines. With the disruption to the myofibrillar ultrastructure, it is not surprising that HDP causes an increase in MFI values. MFI quantifies the extent of myofibril fragmentation upon homogenization and is highly correlated to meat tenderness (Olson *et al.* 1977; Culler *et al.* 1978; Whipple *et al.* 1990). The current data confirm findings that MFI values increase with the proteolysis and tenderness improvements associated with meat aging (Taylor *et al.* 1995). In the current study, HDP increased the MFI values only 10%, but decreased WBSF 23% compared to controls at 8 days of aging. This apparent discrepancy in the magnitude of the HDP effects suggests that the instantaneous physical disruption of the myofibrils does not fully account for the tenderizing effect of HDP.

One study utilizing pork loins found that tenderness did not improve on the day of HDP treatment, but that HDP-treated samples were more tender than the controls following aging (Callahan *et al.* 2002). Practically, these results indicate that HDP may be a useful tool for tenderizing muscle cuts that normally undergo limited proteolysis and tenderization with aging. With regard to the mechanism of tenderization, these results would suggest that in addition to an instantaneous tenderization effect, HDP may also influence tenderness through aging-related myofibrillar protein proteolysis. Other studies have shown that high-pressure treatments can enhance proteolytic meat tenderization. Studies utilizing high-hydrostatic-pressure (HHP) processing have shown that high pressure can fragment sarcomere structure (Suzuki *et al.* 1990), disrupt sarcoplasmic reticulum membranes (Elgasim and Kennick 1982), influence calpastatin and calpain activities (Koohmaraie *et al.* 1984b; Homma *et al.* 1995) and cause a release of lysosomal enzymes (Elgasim and Kennick 1982). Thus, it can be postulated that HDP may enhance aging tenderization by influencing protease activity, substrate availability or sarcoplasmic Ca^{2+} levels.

Only a few studies have investigated the direct effect of HDP on specific proteins related to tenderness and other quality attributes. Spanier and Romanowski (2000) reported that HDP causes a decrease in the protein content of the myofibrillar fraction and an increase in the protein content of the sarcoplasmic fraction with subcellular fractionation by differential centrifugation. This study suggested that the redistribution of proteins with HDP

treatment may be caused by changes in protein solubility and protein degradation. Data from Bowker *et al.* (2007) demonstrated that HDP caused slight shifts in the electrophoretic profile of myofibrillar proteins isolated from Brahman top rounds, and suggested that HDP may influence myofibrillar and sarcoplasmic protein solubility. It was postulated that HDP-induced protein degradation could have altered protein solubility. Several studies using HHP processing have shown that high-pressure treatments increase myofibrillar protein solubility in meat homogenates and extracted myofibrils (Macfarlane 1974; Macfarlane and McKenzie 1976). In the current study, HDP treatment of beef strip loins increased the myofibrillar protein solubility (Table 1). The lack of change in the total protein solubility (myofibrillar + sarcoplasmic) with HDP treatment despite changes in myofibrillar protein solubility was likely because of slight decreases in the sarcoplasmic protein solubility with HDP treatment (data not shown). Thus, data from the current study support the argument that the redistribution of proteins in the Spanier and Romanowski (2000) study was partially caused by HDP-induced changes in protein solubility. Although protein solubility changes were not highly correlated to tenderness (Table 3), increases in myofibrillar protein solubility indicate that HDP may have beneficial effects on protein functionality for further processed meat products.

In the current study, SDS-PAGE and Western blotting techniques were used to determine if HDP directly caused muscle protein degradation or influenced aging-related proteolysis. These techniques have been widely used to characterize proteolysis related to aging tenderization. The postmortem degradation of key myofibrillar and cytoskeletal proteins such as titin, nebulin, vinculin, filamin, desmin and TnT have been investigated in order to decipher their role in meat tenderness (Fritz and Greaser 1991; Koohmaraie *et al.* 1991, 1996; Uytterhaegen *et al.* 1992, 1994; Huff-Lonergan *et al.* 1995, 1996; Ho *et al.* 1996). The gel system utilized in the current study was optimized to investigate the effects of HDP and aging on proteins ranging from approximately 15 to 200 kDa in size.

Overall, the data indicate that aging has a more pronounced effect on the electrophoretic profile of muscle proteins than HDP (Fig. 2 and Table 2). Several proteins (MHC, actin, MLC and TnC), however, were not influenced by either HDP or aging treatments. The lack of contractile protein degradation in this study was not surprising given the documented resistance of contractile proteins to postmortem degradation (Yates *et al.* 1983; Bandman and Zdanis 1988) and the fact that HDP has been shown to have limited effects on the ultrastructural integrity of the thick filament (Zuckerman and Solomon 1998). Several protein bands exhibited significant aging effects, but were not influenced by HDP in the current study. The small aging-related increases of the 160- and 140-kD bands, likely M-protein and C-protein, respectively, could

have been the result of degradation products from larger proteins migrating to these positions. A similar phenomenon could explain the slight increase in the intensity of the Tm band between 0 and 5 days of aging. The decrease in the intensity of the desmin and TnI bands between 0 and 5 days of aging treatments was consistent with past data in which desmin (Hwan and Bandman 1989; Ho *et al.* 1996) and TnI (Ho *et al.* 1996) proteins exhibited degradation with postmortem aging. While the aforementioned changes in many of these protein bands were statistically significant, their correlations to tenderness were fairly low (Table 3).

Further research is needed to explain the changes observed in the 95–110 kDa protein range in the current experiment. Despite numerous studies showing that the amount of intact α-actinin does not significantly change with postmortem aging (Hwan and Bandman 1989; Uytterhaegen *et al.* 1992; Ho *et al.* 1996), the α-actinin band observed in the current study showed a slight increase in intensity between 0 and 5 days of aging. It can be postulated that the slight increase in this band could be the result of increased solubility or release of α-actinin from the Z-line with aging. Consistent with the observations of Koohmaraie *et al.* (1984a), an increase in the intensity of the 95-kDa band (data not shown) and the appearance of a 110-kDa band were observed with aging in many of the samples. The 110-kDa protein is thought to be the degradation product of a larger protein (O'Halloran *et al.* 1997). The HDP-induced increase in the 100–110 kDa band observed in a previous study (Bowker *et al.* 2007) was not evident in the current study. This is largely attributed to differences in the time postmortem at which the samples were HDP treated (day 2 versus day 11 or 12 postmortem) and the origin of the muscle samples (strip loins from Angus cross cattle versus top rounds from Brahman cattle).

The most pronounced protein degradation that was observed in the current study was that of TnT. Both HDP and aging had a significant impact on the disappearance of intact TnT and the appearance of a 30-kDa TnT fragment. SDS-PAGE data demonstrated that the abundance of intact TnT decreased with aging and that the overall abundance of intact TnT was lower in HDP-treated samples compared to the controls (Table 2). Conversely, the abundance of the 30-kDa TnT fragment increased with aging and was higher in HDP-treated samples after 0, 5 and 8 days of aging (Table 2). Analyzing the myofibril samples using 15% gels (Fig. 3) and Western blots with TnT-specific antibody (Fig. 4) provided easier visualization of the TnT degradation and confirmed the identity of the 30-kDa fragment. The blots confirm both the HDP and aging effects on the disappearance of intact TnT and the accumulation of the 30-kDa TnT fragment (Table 5). It should be noted that in both the gels and blots, the intact TnT migrated as a closely spaced doublet band. Multiple TnT bands have previously been observed in bovine muscle prior to

aging (Ho *et al.* 1994; Huff-Lonergan *et al.* 1996) and are the result of fast and slow TnT isoforms that have been identified in skeletal muscle (Briggs *et al.* 1990; Malhotra 1994; Muroya *et al.* 2004). For the data analysis in the current study, TnT band intensity values represent the sum intensity of both bands of the doublet.

Data from the current study regarding the degradation of TnT and the accumulation of a 30-kDa TnT fragment confirm those of past studies involving aging tenderization. Researchers have repeatedly shown that a 30-kDa degradation product of TnT accumulates with muscle aging and is associated with tenderization (MacBride and Parrish 1977; Olson *et al.* 1977; Penny and Dransfield 1979; Ho *et al.* 1994; Wheeler and Koohmaraie 1994; Huff-Lonergan *et al.* 1996). Thus, it is not surprising that compared to the other proteins quantified using SDS-PAGE, the 30-kDa TnT fragment exhibited the highest correlation with WBSF measurements across both treatments and aging periods (Table 3). The presence of the 30-kDa fragment at day 0 control samples indicates that some proteolysis had occurred within the first 48 h postmortem prior to HDP treatment. The predictive nature of the 30-kDa fragment is illustrated by the fact that it was the only protein in which the intensity of the band at day 0 samples was correlated to WBSF measurements after 0, 5 and 8 days of aging.

Rather than having a direct structural impact on tenderness, degradation of TnT and accumulation of the 30-kDa fragment are often thought to be merely indicators of the overall postmortem proteolysis associated with aging. Evaluation of day 0 samples in the current study indicates that HDP simultaneously caused both tenderization and a greater accumulation of the 30-kDa TnT fragment compared to controls. This nonaging-related accumulation of the 30-kDa fragment with HDP tenderization may indicate that degradation of troponin may have a direct structural impact on tenderness.

Given that ultrastructural studies indicate HDP fragments the I-band region in sarcomeres (Zuckerman and Solomon 1998), it is not surprising that the HDP-induced protein degradation observed in the current study primarily occurred in proteins associated with the thin filament. Coupled with the strong tenderness improvements, the lack of significant HDP by aging interaction effects and the limited proteolysis in this study seem to suggest that HDP tenderization is primarily caused by the instantaneous physical fragmentation of the myofibrils. The influence of HDP on the extent of TnT degradation, however, suggests for the first time that HDP may enhance tenderness by instantaneous degradation of proteins associated with tenderness and by enhancing aging-related proteolysis.

The tenderization effect of HDP may be caused by the influence that high-energy shock waves have on high-molecular-weight proteins that are known to maintain the structural integrity of myofibrils. The degradation of

proteins such as titin and nebulin has been associated with the tenderization and proteolysis that occur with postmortem aging (Huff-Lonergan *et al.* 1995, 1996; Taylor *et al.* 1995), but was not investigated in the current experiment. Although bands corresponding to these proteins were observed in many of the SDS-PAGE profiles from the current study, the gel system was not optimized to maximize band separation in this molecular weight range. Thus, further research is needed to determine the effects of HDP on high-molecular-weight proteins related to tenderness.

CONCLUSION

Data from this study demonstrate that HDP is an effective postharvest technique for enhancing tenderness in fresh beef cuts. HDP treatment of beef strip loins caused instantaneous tenderness improvements that were further enhanced by postmortem aging. HDP-treated beef underwent accelerated aging tenderization and achieved levels of tenderness not reached by aging alone. Furthermore, control samples required 8 days of aging to attain the level of tenderness achieved by "nonaged" HDP-treated day 0 samples. Data demonstrating that HDP treatment influenced TnT degradation suggest that in addition to the instantaneous ultrastructural effect on the myofibrillar apparatus, HDP may have a proteolytic, aging-related effect on beef tenderness.

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REFERENCES

AMSA. 1995. *Research Guidelines for Cookery, Sensory Evaluation, and Instrumental Tenderness of Fresh Meat*, American Meat Science Association and National Livestock and Meat Board, Chicago, IL.

BANDMAN, E. and ZDANIS, D. 1988. An immunological method to assess protein degradation in post-mortem muscle. Meat Sci. *22*, 1–19.

BERRY, B.W., SOLOMON, M.B., JOHNSON, W.L., LONG, J.B., EASTRIDGE, J.S. and ZUCKERMAN, H. 1997. Application of the Hydrodyne process to strip loins from U.S. Select grade beef. J. Anim. Sci. *75*(Suppl. 1), 128.

BOWKER, B.C., LIU, M.N., SOLOMON, M.B., EASTRIDGE, J.S., FAHRENHOLZ, T.M. and VINYARD, B. 2007. Effects of hydrodynamic pressure processing and blade tenderization on intramuscular collagen and tenderness-related protein characteristics of top rounds from Brahman cattle. J. Muscle Foods *18*, 35–55.

BRIGGS, M.M., MCGINNIS, H.D. and SCHACHAT, F. 1990. Transitions from fetal to fast troponin-T isoforms in developing rabbit skeletal muscle. Dev. Biol. *140*, 253–260.

CALLAHAN, J.A., SOLOMON, M.B., PAROCZAY, E.W., EASTRIDGE, J.S., PURSEL, V.G. and MITCHELL, A.D. 2002. Enhancement of pork quality using the Hydrodynamic pressure process. In *2002 IFT Annual Meeting Book of Abstracts*, p. 111, Institute of Food Technologists, Anaheim, CA.

CLAUS, J.R., SCHILLING, J.K., MARRIOTT, N.G., DUNCAN, S.E., SOLOMON, M.B. and WANG, H. 2001. Hydrodynamic shock wave tenderization effects using a cylinder processor on early deboned broiler breasts. Meat Sci. *58*, 287–292.

CULLER, R.D., PARRISH, F.C., SMITH, G.C. and CROSS, H.R. 1978. Relationship of myofibril fragmentation index to certain chemical, physical and sensory characteristics of bovine *longissimus* muscle. J. Food Sci. *43*, 1177–1180.

DAVEY, C.L. and DICKSON, M.R. 1970. Studies in meat tenderness. 8. Ultra-structural changes in meat during aging. J. Food Sci. *35*, 56–60.

ELGASIM, E.A. and KENNICK, W.H. 1982. Effect of high hydrostatic pressure on meat microstructure. Food Microstructure *1*, 75–82.

FRITZ, J.D. and GREASER, M.L. 1991. Changes in titin and nebulin in postmortem bovine muscle revealed by gel electrophoresis, Western blotting and immunofluorescence microscopy. J. Food Sci. *56*, 607–610.

GOLL, D.E., YOUNG, R.B. and STROMER, M.H. 1974. Separation of subcellular organelles in differential and density gradient centrifugation. In *Proceedings of 27th Reciprocal Meat Conference*, pp. 250–290, American Meat Science Association, Chicago, IL.

GORNALL, A.G., BARDAWILL, C.J. and DAVID, M.M. 1949. Determination of serum proteins by means of the biuret reaction. J. Biol. Chem. *177*, 751–766.

HO, C.Y., STROMER, M.H. and ROBSON, R.M. 1994. Identification of the 30 kDa polypeptide in postmortem skeletal muscle as a degradation product of troponin-T. Biochimie *76*, 369–375.

HO, C.Y., STROMER, M.H. and ROBSON, R.M. 1996. Effect of electrical stimulation on postmortem titin, nebulin, desmin, and troponin-T degradation and ultrastructural changes in bovine *longissimus* muscle. J. Anim. Sci. *74*, 1563–1575.

HOMMA, N., IKEUCHI, Y. and SUZUKI, A. 1995. Levels of calpain and calpastatin in meat subjected to high pressure. Meat Sci. *41*, 251–260.

HOPKINS, D.L., LITTLEFIELD, P.J. and THOMPSON, J.M. 2000. A research note on factors affecting the determination of myofibrillar fragmentation. Meat Sci. *56*, 19–22.

HUFF-LONERGAN, E., PARRISH, F.C. and ROBSON, R.M. 1995. Effects of postmortem aging time, animal age, and sex on degradation of titin and nebulin in bovine *longissimus* muscle. J. Anim. Sci. *73*, 1064–1073.

HUFF-LONERGAN, E., MITSUHASHI, T., BEEKMAN, D.D., PARRISH, F.C., OLSON, D.G. and ROBSON, R.M. 1996. Proteolysis of specific muscle structural proteins by μ-calpain at low pH and temperature is similar to degradation in postmortem bovine muscle. J. Anim. Sci. *74*, 993–1008.

HUFFMAN, K.L., MILLER, M.F., HOOVER, L.C., WU, C.K., BRITTIN, H.C. and RAMSEY, C.B. 1996. Effect of beef tenderness on consumer satisfaction with steaks consumed in the home and restaurant. J. Anim. Sci. *74*, 91–97.

HWAN, S. and BANDMAN, E. 1989. Studies of desmin and α-actinin degradation in bovine *semitendinosus* muscle. J. Food Sci. *54*, 1426–1430.

KOOHMARAIE, M., KENNICK, W.H., ANGLEMIER, A.F., ELGASIM, E.A. and JONES, T.K. 1984a. A research note: Effect of postmortem storage on cold-shortened bovine muscle: Analysis by SDS-polyacrylamide gel electrophoresis. J. Food Sci. *49*, 290–291.

KOOHMARAIE, M., KENNICK, W.H., ELGASIM, E.A. and ANGLEMIER, A.F. 1984b. Effect of prerigor pressurization on the activity of calcium-activated factor. J. Food Sci. *49*, 680–684.

KOOHMARAIE, M., WHIPPLE, G., KRETCHMAR, D.H., CROUSE, J.D. and MERSMANN, H.J. 1991. Postmortem proteolysis in *longissimus* muscle from beef, lamb, and pork carcasses. J. Anim. Sci. *69*, 617–624.

KOOHMARAIE, M., DOUMIT, M.E. and WHEELER, T.L. 1996. Meat toughening does not occur when rigor shortening is prevented. J. Anim. Sci. *74*, 2935–2942.

LIU, M.N., SOLOMON, M.B., VINYARD, B., CALLAHAN, J.A., PATEL, J.R., WEST, R.L. and CHASE, C.C. 2006. Use of hydrodynamic pressure processing and blade tenderization to tenderize top rounds from Brahman cattle. J. Muscle Foods *17*, 79–91.

LOCKER, R.H. 1960. Degree of muscular contraction as a factor in tenderness of beef. Food Res. *25*, 304–307.

MACBRIDE, M.A. and PARRISH, F.C. 1977. The 30,000-dalton component of tender bovine *longissimus* muscle. J. Food Sci. *42*, 1627–1629.

MACFARLANE, J.J. 1974. Pressure-induced solubilization of meat proteins in saline solution. J. Food Sci. *39*, 542–547.

MACFARLANE, J.J. and MCKENZIE, I.J. 1976. Pressure-induced solubilization of myofibrillar proteins. J. Food Sci. *41*, 1442–1446.

MALHOTRA, A. 1994. Role of regulatory proteins (troponin–tropomyosin) in pathologic states. Mol. Cell. Biochem. *135*, 43–50.

MARRIOTT, N.G., WANG, H., SOLOMON, M.B. and MOODY, W.G. 2001. Studies of cow beef tenderness enhancement through supersonic–hydrodynamic shock wave treatment. J. Muscle Foods *12*, 207–218.

MARSH, B.B. and LEET, N.G. 1966. Studies in meat tenderness. III. The effects of cold shortening on tenderness. J. Food Sci. *31*, 450–459.

MILLER, M.F., CARR, M.F., RAMSEY, C.B., CROCKETT, K.L. and HOOVER, L.C. 2001. Consumer thresholds for establishing the value of beef tenderness. J. Anim. Sci. *79*, 3062–3068.

MOELLER, S., WULF, D., MEEKER, D., NDIFE, M., SUNDARARAJAN, N. and SOLOMON, M.B. 1999. Impact of the Hydrodyne process on tenderness, microbial load, and sensory characteristics of pork *longissimus* muscle. J. Anim. Sci. *77*, 2119–2123.

MUROYA, S., KITAMURA, S., TANABE, S., NISHIMURA, T., NAKAJIMA, I. and CHIKUNI, K. 2004. N-terminal amino acid sequences of troponin T fragments, including 30 kDa one, produced during postmortem aging of bovine *longissimus* muscle. Meat Sci. *67*, 19–24.

O'HALLORAN, G.R., TROY, D.J. and BUCKLEY, D.J. 1997. The relationship between early postmortem pH and the tenderization of beef muscles. Meat Sci. *45*, 239–251.

OLSON, D.G., PARRISH, F.C., DAYTON, W.R. and GOLL, D.E. 1977. Effect of postmortem storage and calcium activated factor on the myofibrillar proteins of bovine skeletal muscle. J. Food Sci. *42*, 117–124.

PENNY, I.F. and DRANSFIELD, E. 1979. Relationship between toughness and troponin-T in conditioned beef. Meat Sci. *3*, 135–141.

SCHILLING, M.W., CLAUS, J.R., MARRIOTT, N.G., SOLOMON, M.B., EIGEL, W.N. and WANG, H. 2002. No effect of hydrodynamic shock wave on protein functionality of beef muscle. J. Food Sci. *67*, 335–340.

SOLOMON, M.B. 1987. A research note: Comparison of methods for measuring pH in muscle tissue. J. Food Sci. *52*, 1428–1429.

SOLOMON, M.B., LONG, J.B. and EASTRIDGE, J.S. 1997. The Hydrodyne: A new process to improve beef tenderness. J. Anim. Sci. *75*, 1534–1537.

SOLOMON, M.B., CARPENTER, C.E., SNOWDER, G.D. and COCKETT, N.E. 1998. Tenderizing callipyge lamb with the Hydrodyne process and electrical stimulation. J. Muscle Foods *9*, 305–311.

SOLOMON, M.B., BERRY, B.W., PAROCZAY, E.W., CALLAHAN, J.A. and EASTRIDGE, J.S. 2002. Effects of hydrodynamic pressure processing (HDP) and aging on beef tenderness. In *2002 IFT Annual Meeting Book of Abstracts*, p. 111, Institute of Food Technologists, Anaheim, CA.

SOLOMON, M.B., LIU, M.N., PATEL, J.R., BOWKER, B.C. and SHARMA, M. 2006. Hydrodynamic pressure processing to improve meat quality and safety. In *Advanced Technologies for Meat Processing* (L.M.L. Nollet and F. Toldra, eds.) pp. 219–244, CRC Press Taylor & Francis Group, Boca Raton, FL.

SPANIER, A.M. and ROMANOWSKI, R.D. 2000. A potential index for assessing the tenderness of hydrodynamic pressure (HDP)-treated beef strip loins. Meat Sci. *56*, 193–202.

SUZUKI, A., WATANABE, M., IWAMURA, K., IKEUCHI, Y. and SAITO, M. 1990. Effect of high pressure treatment on the ultrastructure and myofibrillar protein of beef skeletal muscle. Agric. Biol. Chem. *54*, 3085–3091.

TAYLOR, R.G., GEESINK, G.H., THOMPSON, V.F., KOOHMARAIE, M. and GOLL, D.E. 1995. Is Z-disk degradation responsible for postmortem tenderization? J. Anim. Sci. *73*, 1351–1367.

UYTTERHAEGEN, L., CLAEYS, E. and DEMEYER, D. 1992. The effect of electrical stimulation on beef tenderness, protease activity and myofibrillar protein fragmentation. Biochimie *74*, 275–281.

UYTTERHAEGEN, L., CLAEYS, E. and DEMEYER, D. 1994. Effects of exogenous protease effectors on beef tenderness development and myofibrillar degradation and solubility. J. Anim. Sci. *72*, 1209–1223.

WHEELER, T.L. and KOOHMARAIE, M. 1994. Prerigor and postrigor changes in tenderness of bovine *longissimus* muscle. J. Anim. Sci. *72*, 1232–1238.

WHIPPLE, G., KOOHMARAIE, M., DIKEMAN, M.E. and CROUSE, J.D. 1990. Predicting beef-*longissimus* tenderness from various biochemical and histological muscle traits. J. Anim. Sci. *68*, 4193–4199.

YATES, L.D., DUTSON, T.R., CALDWELL, J. and CARPENTER, Z.L. 1983. Effect of temperature and pH on the postmortem degradation of myofibrillar proteins. Meat Sci. *9*, 157–179.

ZUCKERMAN, H. and SOLOMON, M.B. 1998. Ultrastructural changes in bovine *longissimus* muscle caused by the Hydrodyne process. J. Muscle Foods *9*, 419–426.