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BLADE TENDERIZATION AND HYDRODYNAMIC PRESSURE PROCESSING EFFECTS ON PROTEIN CHARACTERISTICS IN TOP ROUNDS FROM BRAHMAN CATTLE

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Introduction

Inconsistent beef tenderness is a major problem in the meat industry. Due to the high consumer demand for lean yet tender cuts of beef and the high degree of tenderness variability between muscles and animals, it is necessary for the meat industry to develop methodologies to effectively tenderize tough cuts of beef. Technologies such as blade tenderization (BT) and hydrodynamic pressure processing (HDP) can be utilized to improve the tenderness of inherently tough cuts of meat, such as those from Brahman cattle. BT physically tenderizes muscle by inserting blades or needle probes into the muscle tissue to sever connective tissue and the myofibrillar structure. HDP has been shown to reduce shear force values by 59% in cold shortened beef semimembranosus muscles (Solomon et al., 1997), however, the mechanism by which HDP shock waves tenderize meat is not fully understood. HDP tenderization is thought to occur through the physical disruption of the myofibrillar structure (Zuckerman and Solomon, 1998). It was hypothesized that combining BT and HDP would greatly enhance the tenderness of tough beef cuts compared to either treatment alone due to BT disrupting the intramuscular connective tissue and allowing more extensive HDP tenderization (Liu et al., 2004). Although previous work has established that BT and HDP technologies effectively tenderize meat, there is a lack of data characterizing the effects that these technologies have on muscle proteins as they relate to muscle ultrastructure and the mechanism of tenderization.

Objectives

The objective of this study was to investigate the effects of HDP, BT, and BT followed by HDP (BT+HDP) on protein characteristics related to tenderness and protein functionality of inherently tough top rounds taken from Brahman cattle.

Methodology

Top rounds from Brahman cattle (Sub Tropical Agriculture Research Station, Brookville, FL) were frozen 7 days postmortem. Tenderness prescreening was conducted and twelve of the toughest top rounds (shear force range: 6.80 – 9.75 kgf) were selected for this study. Top rounds were thawed (2°C for 96-120 h) and subdivided parallel to the

long axis of the cut into two sections. Each section was randomly assigned to one of three treatments: BT, HDP, or BT+HDP in a balanced incomplete block design. Prior to treatment a subsection was removed perpendicular to the fiber direction of the muscles within each section to serve as a paired control. BT and BT+HDP samples were passed once through a blade tenderizer unit (Model MT-M5, Lumar Ideal Inc., Montreal, Quebec, Canada) parallel to the muscle fibers (2.3 penetrations/cm^2). HDP and BT+HDP samples were vacuum packaged, heat shrunk, and placed on a flat steel reflector plate inside a 98-L suspended plastic container filled with water. A 100 g binary explosive, detonated 31cm above the meat, was used to generate the hydrodynamic pressure.

Following the tenderization treatments, 2.5 cm thick steaks were cut from each subsection (both treated and control) and cooked to 71°C according to the AMSA (1995) guidelines on an electric George Foreman Indoor/Outdoor grill (Model GGR50B, Salton, Mount Prospect, IL). Warner-Bratzler shear force (kgf) measurements were obtained using the Universal Instron Testing Machine (Model 1122, Instron Corporation, Canton, MA).

In addition to removing steaks for tenderness evaluation following the tenderization treatments, samples were also removed for protein analysis. These samples were vacuum packaged, stored at -20°C, and thawed overnight at 4°C prior to processing and analysis. From these samples, the sarcoplasmic and myofibrillar protein fractions were isolated by homogenization/centrifugation according to the procedure of Goll et al. (1974) with modifications. Isolated protein fractions were separated electrophoretically using the SDS-PAGE technique of Laemmli (1970) with modifications. Myofibrillar proteins were separated on 4-20% acrylamide gradient gels and sarcoplasmic proteins were separated on 10-20% acrylamide gradient gels. Samples were dissolved in sample buffer (8.0 M Urea, 2.0 M Thiourea, 0.05 M Tris (pH 6.8), 75 mM DTT, 3 % SDS, 0.05 % Bromophenol blue) in boiling water for 5 minutes. Bands were visualized by Coomassie brilliant blue R-250 staining and the intensity of the bands was measured using a KODAK Gel Logic 200 imaging system and KODAK 1D Image Analysis software (Eastman KODAK Co., Rochester, NY). Myofibrillar fragmentation index (MFI) was measured for each sample according to the procedure of Hopkins et al. (2000). Sarcoplasmic protein solubility was determined in 0.025 M potassium phosphate (pH 7.2) buffer and myofibrillar protein solubility was similarly determined in 1.1 M KI/0.1 M potassium phosphate (pH 7.2) buffer according to the procedures of Schilling et al. (2002). All protein concentrations were measured using the biuret method (Total protein reagent, Sigma-Aldrich Inc., St. Louis, MO).

MFI, protein solubility, and SDS-PAGE band intensity data were analyzed using the Proc MIXED procedure in SAS® (version 9.1, SAS Institute Inc., Cary, NC). The model included treatment (BT, HDP, BT+HDP) and type (control, treated) as class independent variables and round as a random effect.

Results & Discussion

A previous study on these samples demonstrated that BT and HDP improved tenderness 18% and that BT+HDP improved tenderness 14% compared to paired controls (Liu et al., 2004). Table 1 shows the impact tenderization treatments had on MFI, protein solubility, and SDS-PAGE banding patterns. The MFI value, a measure of the degree of

fragmentation of myofibrils upon homogenization, is often used as an indicator of the degradation of myofibrillar proteins under postmortem conditions. Overall, MFI values were negatively correlated to shear force measurements ($r = -0.53$). 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. Thus, MFI data suggest that part of the tenderization effect of BT and HDP treatments is the result of a breakdown of intra-and intermyofibril linkages.

Past research has shown that high pressure treatments can influence the solubility characteristics of various muscle proteins (Macfarlane and McKenzie, 1976). This phenomenon, however, was not readily apparent with the HDP and BT+HDP treatments. Neither sarcoplasmic nor myofibrillar protein solubility differed statistically ($p>0.05$) between treated and control samples. Sarcoplasmic and myofibrillar protein solubility did not significantly 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 rounds (data without these samples not shown). Due to the small number of samples, a large decrease in the myofibrillar protein solubility between the control and treated samples taken from these two rounds contributed to the high degree of variability in the myofibrillar solubility measurements and masked overall treatment effects. Overall, solubility data did not significantly correlate with tenderness data.

To investigate shifts in protein profiles and proteolytic differences between samples, SDS-PAGE analysis was performed. Sarcoplasmic electrophoretic protein gels exhibited banding pattern differences between top rounds (particularly 185, 145, 90, 42, and 23.5 kDa fragments), but minimal differences between paired control and treatment samples. Likewise, few differences were observed among BT, HDP, and BT+HDP treatments with respect to sarcoplasmic protein banding patterns. Myofibrillar protein banding patterns also varied significantly between top round samples. Compared to paired controls, HDP and BT+HDP samples had significantly higher ($p<0.05$) ~100-110 kDa to actin band intensity ratios (Table 1), whereas BT and control samples did not differ. The identity of this band has not been positively determined but it is hypothesized to be part of the C-protein (~140 kDa) which helps stabilize myosin molecules in the thick filament. This hypothesis is based on the findings of O'Halloran et al (1997) that during aging of bovine muscle a 110 kDa fragment appeared in the myofibrillar fraction which was later shown by sequence analysis to have strong homology with C-protein (Casserly et al., 1998). The myofibrillar electrophoretic protein gels also showed a considerable amount of variation in the intensity of the 30 kDa protein fragment from sample to sample, but no treatment differences were detectable.

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 or additive 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 few if any protein solubility or SDS-PAGE banding pattern differences compared to controls confirms that BT likely tenderizes muscle by physically disrupting and severing the connective tissue

and myofibrillar structure. Slight shifts in the SDS-PAGE banding patterns of myofibrillar proteins and increased MFI with HDP and BT+HDP treatment indicate that in addition to the physical disruption to the myofibrillar structure, HDP treatment may also influence tenderization through direct alterations of muscle proteins.

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Tables and Figures

Table 1. Least square means ± standard errors of MFI, protein solubility, and SDS-PAGE myofibrillar protein band intensity measurements of blade tenderization (BT), hydrodynamic pressure processing (HDP), BT followed by HDP (BT+HDP), and control samples from Brahman top rounds.

	Type		Significance		
Treatment	Control	Treated	Trt[1]	Type[2]	Trt × Type
Myofibrillar Fragmentation Index					
BT	45.8 ± 6.3[b]	62.2 ± 8.2[a]	0.9844	0.0020	0.9934
HDP	46.5 ± 7.0[b]	62.7 ± 6.9[a]			
BT+HDP	45.8 ± 6.7[b]	61.4 ± 5.9[a]			
Sarcoplasmic Protein Solubility (mg protein/g muscle tissue)					
BT	41.2 ± 2.9	38.3 ± 2.1	0.6631	0.1796	0.7714
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	0.4737	0.8239	0.1917
HDP	134.3 ± 13.6	127.4 ± 12.8			
BT+HDP	111.3 ± 9.1	127.1 ± 11.1			
Myofibrillar protein SDS-PAGE staining intensity (100-110 kDa : actin band intensity ratio)					
BT	0.181 ± 0.015[b]	0.191 ± 0.022[ab]	0.0863	0.0216	0.4631
HDP	0.185 ± 0.010[b]	0.229 ± 0.015[a]			
BT+HDP	0.176 ± 0.007[b]	0.209 ± 0.008[a]			

[1] Trt factor compares BT, HDP, and BT+HDP

[2] Type factor compares Control to Treated samples

[a,b] Values with different superscripts differ significantly ($p<0.05$)