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Microstructure alterations in beef intramuscular connective tissue caused by hydrodynamic pressure processing☆

H. Zuckerman [a,1], B.C. Bowker [b,*], J.S. Eastridge [c], M.B. Solomon [c]

[a] USDA Agricultural Research Service, 10300 Baltimore Ave., Bldg. 201 BARC-East, Beltsville, MD 20705, USA
[b] USDA Agricultural Research Service, 950 College Station Rd., Athens, GA 30605, USA
[c] USDA Agricultural Research Service, 10300 Baltimore Ave., Bldg. 209 BARC-East, Beltsville, MD 20705, USA

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ABSTRACT

Scanning electron microscopy (SEM) was utilized to evaluate microstructural changes in intramuscular connective tissue of beef *semimembranosus* muscle subjected to hydrodynamic pressure processing (HDP). Samples were HDP treated in a plastic container (HDP-PC) or a steel commercial unit (HDP-CU). Control and HDP samples were obtained immediately post-treatment and after 14 days of aging for SEM and Warner–Bratzler shear force (WBSF) analysis. Immediately post-treatment, HDP treated samples exhibited lower ($P < 0.01$) WBSF than did controls. After aging, HDP-PC samples had lower ($P < 0.01$) WBSF than that of aged controls. SEM analysis indicated that HDP-PC treatment disrupted the integrity of the collagen fibril network of the endomysium in both the non-aged and aged samples. Aging effects on the intramuscular connective tissue were observed in the HDP-PC and control samples. Both WBSF and connective tissue changes were greater in the HDP-PC than in the HDP-CU treated samples. Data suggest that shockwave alterations to connective tissue contribute to the meat tenderization of HDP.

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1. Introduction

Hydrodynamic pressure processing (HDP) is a novel postharvest technology for improving the tenderness of fresh meat cuts. HDP utilizes high pressure shockwaves generated by small amounts of high energy explosives to tenderize vacuum-packaged meat submerged in water. Past data has shown that HDP tenderizes fresh cuts of beef from the loin and round (Solomon, Long, & Eastridge, 1997) with minimal impact on fresh meat color and water-holding capacity. The degree of meat tenderization with HDP application depends on various parameters of the HDP system such as the type, quantity, shape, and placement of the explosive used to generate the shockwave, as well as the configuration and composition of the containment vessel (Solomon, Liu, Patel, Bowker, & Sharma, 2006). Inherent differences within the muscle, such as the extent of muscle shortening prior to rigor onset, muscle fiber orientation, and postmortem aging time prior to HDP application also impact HDP tenderization (Solomon et al., 2006). While many of the methodological factors that influence HDP tenderization have been well-defined, the impact of high pressure shockwaves on muscle ultrastructure and various components of muscle tissue are not well understood. In order to further advance HDP technology for improving meat quality, additional data is needed to understand the biological basis of shockwave induced meat tenderization.

Meat tenderness is influenced by both the myofibrillar and connective tissue components of muscle tissue. Past research has demonstrated that high pressure HDP shockwaves alter the myofibrils of muscle by directly causing physical disruption to the sarcomere structure. Zuckerman and Solomon (1998) demonstrated that HDP treatment of beef *longissimus* muscle caused fragmentation within the I-band region of sarcomeres, disrupted Z-line integrity, and increased spacing between myofibrils. Furthermore, the enzymatic degradation of myofibrillar proteins with aging tenderization was enhanced in beef *longissimus* muscle that was HDP treated prior to aging (Bowker, Fahrenholz, Paroczay, Eastridge, & Solomon, 2008).

The effect of HDP on the connective tissue component of muscle is not well understood. Both the total amount of intramuscular connective tissue and the degree of collagen cross-linking within connective tissue impact meat tenderness. Marriott, Wang, Solomon, and Moody (2001) found that HDP treatment did not influence the amount or solubility of intramuscular collagen in beef *longissimus* muscles from older cows. In *semimembranosus* muscle from Brahman cattle, however, HDP was found to increase collagen solubility (Bowker et al., 2007). As a means to assess connective tissue, these studies utilized

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* Corresponding author at: USDA Agricultural Research Service, Quality & Safety Assessment Research Unit, 950 College Station Rd., Athens, Georgia 30605, USA. Tel.: +1 706 546 3610; fax: +1 706 546 3607.
E-mail address: brian.bowker@ars.usda.gov (B.C. Bowker).
[1] This author was formerly with the USDA Agricultural Research Service.

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standard heat-labile and acid hydrolysis methods to measure soluble and total muscle collagen biochemically. Based on these differing results, it is hypothesized that any effect of HDP on intramuscular connective tissue may be subtle, varied, and better suited to detection through microscopy techniques. Thus, the objective of this study was to utilize a cell maceration scanning electronic microscopy (SEM) technique to determine the effect of HDP on the microstructure and integrity of intramuscular connective tissue in beef *semimembranosus*.

2. Materials and methods

2.1. Muscle samples and treatments

At 48 h postmortem, *semimembranosus* muscles were excised from both sides of four USDA Select and Choice beef carcasses at a commercial slaughter facility. Muscle samples were immediately vacuum packaged and held at 4 °C until day 7 postmortem. On the day of treatment application, each *semimembranosus* was divided into three sections from the sirloin end. One section of each *semimembranosus* served as a non-treated control, while the other two sections were HDP treated using two different HDP units (plastic container, HDP-PC; commercial unit, HDP-CU). Immediately following treatment application, samples were removed from each *semimembranosus* section (control, HDP-PC, HDP-CU) for shear force and SEM evaluation on the day of treatment (day 7 postmortem). The remaining portion of the control and HDP-PC sections were vacuum packaged and aged at 4 °C for shear force and SEM evaluation on day 21 postmortem.

2.2. Hydrodynamic pressure processing treatments

Hydrodynamic pressure processing (HDP) was performed in either a plastic container (HDP-PC; 208-L volume, 51 cm diameter, 2 mm thick walls) or a stainless steel commercial prototype unit (HDP-CU; 1060-L volume, 120 cm diameter, 7.6 cm thick walls). With the HDP-PC unit, a 2 cm thick steel plate was placed on the bottom of the vessel to reflect the ensuing shockwave back through the meat to intersect the incoming wave. For both HDP treatments, vacuum-packaged meat samples were placed at the bottom of the water-filled vessels and an explosive charge was detonated at a set distance above the sample. For the HDP-PC treatment, 150 g of explosives were detonated 35.6 cm above the surface of the meat. For the HDP-CU treatment, 450 g of explosives were detonated 61.0 cm above the surface of the meat. The parameters of the two HDP treatments were designed to generate similar shockwave pressures (~100 MPa) at the meat surface.

2.3. Warner–Bratzler shear force measurement

Steaks (2.54 cm thick) for Warner–Bratzler shear force (WBSF) analysis were cooked using Farberware Open Hearth broilers (Model 350A, Walter Kidde and Co., Bronx, NY, USA) according to AMSA (1995) guidelines. Internal temperature was monitored using iron–constantan thermocouples attached to a Speedomax® multipoint recording potentiometer (Model 1650, Leeds and Northrup, North Wales, PA, USA) and the steaks were turned midway between initial and endpoint temperatures (4 °C and 71 °C, respectively). After the steaks had been cooled to room temperature, core samples were cut parallel to muscle fiber orientation and peak shear force was measured using a Warner–Bratzler shear test cell mounted on a texture measurement system (Model 1122, Instron Corp., Canton, MA, USA).

2.4. Scanning electron microscopy (SEM)

From each *semimembranosus* portion, 1 × 1 × 1.5 cm samples were excised for evaluating microstructure alterations in intramuscular connective tissue using a cell maceration scanning electron microscopy (SEM) technique (Ohtani, Ushiki, Taguchi, & Kikuta, 1988). The SEM samples were transferred to glass vials containing 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3 at 22 °C and fixed for 1 day. The initial fixation was followed by cellular maceration (Ohtani et al., 1988). Briefly, the samples were immersed in 10% (w/v) NaOH for 7 days then rinsed in distilled water over 5 days at room temperature. The samples were then placed in 1% (w/v) tannic acid for 3 h, rinsed in distilled water for several hours, and post-fixed with 1% osmium tetroxide for 1 h. The specimens were dehydrated in a series of graded concentrations of ethanol, freeze-fractured in liquid nitrogen using a razor blade, and critical point dried using liquid CO_2 to preserve tissue morphology. The dried specimens were mounted on metal stubs with double-stick tape, coated with gold and observed under a scanning electron microscope (Hitachi model S-570, Tokyo, Japan) with an accelerating voltage of 15 kV. Images were calibrated to determine magnification.

2.5. Statistical design and analysis

Shear force data were analyzed as a one-way ANOVA using the PROC MIXED procedure of SAS® Version 9.12 Statistical Analysis System (SAS Institute, Cary, NC, U.S.A.) software. The model included five treatment combinations (control, control + aging, HDP-PC, HDP-PC + aging, HDP-CU) and individual *semimembranosus* muscles as random block effects. Least squares means and standard errors were estimated and significant ($P < 0.05$) differences among means were determined using the PDIFF and SIDAK options for multiple mean comparisons.

3. Results and discussion

The effects of HDP and aging on *semimembranosus* tenderness measurements are shown in Fig. 1. Compared to the non-treated controls, HDP-PC decreased ($p < 0.01$) WBSF by 37% and HDP-CU decreased ($p < 0.01$) WBSF by 25% immediately post-treatment. After aging for 14 additional days, the HDP-PC treated samples exhibited 31% lower WBSF values than did aged controls. With aging WBSF decreased by 28% in the controls and by 20% in the HDP-PC treated samples.

The HDP induced improvements in *semimembranosus* tenderness observed in this study are similar to those from previous research with fresh beef cuts (Solomon et al., 1997). While both HDP treatments reduced WBSF compared to the non-treated controls immediately post-treatment, the degree of meat tenderization was slightly greater in the *semimembranosus* samples treated in the plastic container (HDP-PC) than those in the steel commercial unit (HDP-CU). In the current study, the two different HDP treatments were designed

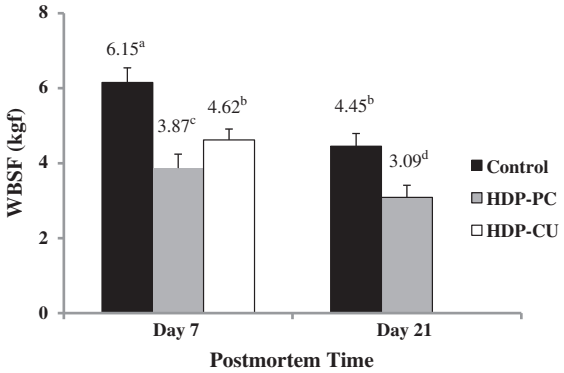

Fig. 1. Warner–Bratzler (WBSF) shear force (means ± standard error) of beef *semimembranosus* muscles: non-treated controls; HDP treatment in plastic container (HDP-PC); HDP treatment in steel commercial unit (HDP-CU). WBSF was measured immediately post-treatment (day 7 postmortem) and after aging (day 21 postmortem). Means with different superscripts differ significantly ($P < 0.05$).