



Relationship Between Meat Quality, Carcass Characteristics, and Protein Abundance of $HSP\beta1$, HSPA, and DJ1 in Beef *Longissimus thoracis* Pre-Rigor or After 14 Days' Aging

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Abstract: This study evaluated associations of heat shock proteins (HSP) and an oxidative stress protein, protein deglycase (DJ1), with beef quality and tenderness. Samples from the *longissimus thoracis* (N = 99) were collected pre-rigor (day 0) and after 14-d aging. Warner-Bratzler shear force (WBSF), myofibrillar fragmentation index (MFI), and a trained sensory panel were used to determine meat quality. Protein abundance of DJ1 and 2 HSP—HSP\(\theta\)1 and HSPA—were assessed. Regression analyses demonstrated that DJ1 abundance after 14 d of aging is a predictor of WBSF (P < 0.001), MFI (P = 0.02), and sensory panel tenderness (P < 0.001). Abundance of HSP β 1 after 14 d of aging is also a predictor of MFI (P = 0.03). Additionally, abundance of both HSP β 1 and DJ1 pre-rigor are predictors of juiciness (P < 0.05). Abundance of HSP β 1 pre-rigor was correlated with WBSF (R = 0.67), sensory panel tenderness (R = -0.44), juiciness (R = -0.30), and umami (R = -0.20). Abundance of DJ1 pre-rigor was also correlated with WBSF (R = 0.72), sensory panel tenderness (R = -0.44), juiciness (R = -0.24), and umami (R = -0.31). After 14-d aging, HSP β 1 abundance was correlated with WBSF (R = 0.66), sensory panel tenderness (R = -0.34), juiciness (R = -0.34), umami (R = -0.33), and brown/ roasted (R = -0.30). Abundance of DJ1 after 14-d aging was also correlated with WBSF (R = 0.68), sensory panel tenderness (R = -0.41), juiciness (R = -0.21), and umami (R = -0.28). These results demonstrate that abundance of HSP β 1 and DJ1 both pre-rigor and after 14 d of aging are correlated with meat tenderness and end-product quality as assessed by a trained sensory panel. Regression analyses further reveal that abundance of DJ1 and HSPβ1 after 14 d of aging is causative in development of beef tenderness and juiciness, respectively. Taken together, these results suggest that abundance of DJ1 is a predictor of tenderness, whereas abundance of HSPβ1 is related to meat quality but cannot be used to predict tenderness.

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Introduction

Consumers consider tenderness to be one of the most important qualities of beef and are willing to pay a premium price for a product with guaranteed tenderness (Miller et al., 2001; Koohmaraie et al., 2002; Laville et al., 2009; Lucero-Borja et al., 2014). However, despite similar production practices, beef cattle exhibit undesirable variation in the rate and extent of postmortem proteolysis leading to

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inconsistencies in tenderness. The process of tenderization occurs during the conversion of muscle to meat as a result of proteolysis of myofibrillar and associated structural proteins within the muscle (Laville et al., 2009). Proteolytic systems, such as calpains, cathepsin, caspases, and the proteasome have each been studied to some extent and are known to play a role in the postmortem tenderization process (Sentandreu et al., 2002; Koohmaraie and Geesink, 2006; Ouali et al., 2013). In addition to proteolysis, several other biological pathways-including muscular structure and contraction, heat shock proteins (HSP), apoptosis, transport, and metabolism—are each related to meat tenderness (D'Alessandro et al., 2012a; Picard et al., 2014; Baldassini et al., 2015; Picard et al., 2015; Lana and Zolla, 2016; Picard and Gagaoua, 2017).

HSP are a family of chaperone proteins that are both constitutively expressed and upregulated by cells and tissues in response to cell stressors and have a homeostatic function refolding denatured proteins and preventing protein aggregation, ultimately protecting the cell from apoptosis (Ritossa, 1962; Tissiéres et al., 1974; Pulford et al., 2008; Balan et al., 2014). HSP have been classified into several different families based on their molecular weight. Two families of HSP that have been found to play a role in meat quality are HSPA (Hsp70) and HSPB (small HSP). HSPA is constitutively expressed, upregulated in response to stress, and is adenosine triphosphate (ATP) dependent (Welch, 1992; Creagh et al., 2000). Members of the HSPB, including HSP27 (HSPβ1), are upregulated in response to stress and are thought to act as molecular chaperones, specifically protecting myofibrillar proteins from degradation during events in which the muscle tissue is stressed, such as postmortem conditions (Mymrikov et al., 2011; Balan et al., 2014; Lomiwes et al., 2014). Similar to HSP, the protein deglycase 1 (DJ1) is thought to be involved in cellular protection from apoptosis (Laville et al., 2009; Longo et al., 2015). To date, several studies have determined that DJ1 abundance is related to meat tenderness (Jia et al., 2009; Guillemin et al., 2011b; Picard et al., 2014; Gagaoua et al., 2015). As such, many studies have investigated the relationship between HSP, DJ1, and development of tenderness (Guillemin et al., 2011a; D'Alessandro et al., 2012b; Picard and Gagaoua, 2017; Rosa et al., 2017; Malheiros et al., 2018; Malheiros et al., 2019; Gagaoua et al., 2020; Ma and Kim, 2020). However, few studies have analyzed the relationship between abundance of HSP and carcass characteristics. Previous research indicates that steers with an increased yield grade have decreased expression of HSPβ1 (Keady et al., 2013). Animals that are more susceptible to stress have less desirable carcass characteristics and increased abundance of several different HSP; however, previous research has not characterized the relationship between HSP abundance and carcass characteristics (King et al., 2006; Ferguson and Warner, 2008; Abhijith et al., 2017; Gagaoua et al., 2017). DJ1 has previously been identified as being involved in quality grade (Thornton et al., 2017) and carcass growth (Picard et al., 2017) but is not known to be related to any other carcass characteristics.

Although a number of previous studies have identified a relationship between HSP and DJ1 abundance and various aspects of meat quality, the relationship between abundance of these anti-apoptotic proteins, carcass characteristics, and meat quality is not completely understood. Additionally, much of the research that has been presented on this topic provides contradictory results, warranting additional research to fully elucidate the relationship between abundance of HSP and DJ1, carcass characteristics, and meat quality. Therefore, the objective of this study was to evaluate the relationship of abundance of HSP and DJ1 with tenderness and meat quality of samples collected from the *longissimus thoracis* (LT) both pre-rigor and after 14 d of aging.

Materials and Methods

Sample collection and preparation

Samples were obtained from the LT of 99 randomly selected cattle finished in the Intermountain West at the same feedlot operation and harvested on 4 separate days at a commercial harvest facility (Hyrum, UT) following USDA guidelines. All cattle were Bos taurus mixed breed cattle between 16 and 20 mo of age and were raised using similar production practices for the area. Within 20 min of exsanguination (0 d), skeletal muscle samples were collected from the left LT pre-rigor. Samples were collected following previously described procedures (Thornton et al., 2017). In brief, a homemade core drilling device was used to collect skeletal muscle samples from between the 11th and 12th ribs. Samples were immediately snap frozen in liquid nitrogen and stored at -80°C until further analysis. The left strip loin (Institutional Meat Purchase Specifications #180) from each animal was obtained during fabrication approximately 24 h after exsanguination and transported to the Utah State University Meats Laboratory (Logan, UT), where it was then wet aged for $14 \, d$ at $4^{\circ}C$. After the 14-d aging period, a sample that was approximately $2 \, g$ in size was cut from the same LT when steaks were fabricated. Each sample was collected from the center of the fourth steak that was fabricated taking care to avoid connective tissues and intramuscular fat depots. This sample was snap frozen in liquid nitrogen and stored at $-80^{\circ}C$ until further analysis was performed.

Carcass characteristics

At harvest, carcass characteristics were obtained from the commercial harvest facility. The carcass characteristics obtained were hot carcass weight, ribeye area (REA), marbling score, quality grade, and yield grade. Marbling score, quality grade, yield grade, and REA were all obtained from camera data provided by the commercial harvest facility.

Western blotting

Western blot analysis was used to detect abundance of HSPβ1, HSPA, and DJ1 in samples collected at both 0 d and 14 d of age. All samples collected from the LT were ground under liquid nitrogen using a mortar and pestle. Total protein was extracted following previously described methods (Thornton et al., 2017). In brief, between 50 and 70 mg of ground tissue was transferred to 15 μ L/mg tissue total protein extraction bu er (50 mM Tris-HCl [pH 7.52], 150 mM NaCl, 1 mM ethylenediaminetetraacetic acid, 1% Tergitol, 0.1% sodium dodecyl sulfate [SDS], and 0.5% sodium deoxycholate). Immediately before use, phosphatase and protease inhibitor cocktail tablets (Roche, Indianapolis, IN) were added to the extraction bu er. Samples were homogenized and placed on a rocking platform for 10 min at 4°C. All samples were centrifuged at $10,000 \times g$ for 10 min at 4°C. The supernatant was removed and stored at -80°C. Samples were then quantified using a Pierce BCA Protein Assay Kit (ThermoFisher Scientific, Pittsburgh, PA) as per the manufacturer's protocol. Samples were diluted to a concentration of 10 µg/µL using extraction buffer, and 30 µL of sample was loaded into each well of the gel. Eight individual samples were run on each gel for each protein analyzed; the same internal standard sample was also run on each gel for each protein to serve as a control between the different gels that were run. A total of 25 different gels were run, and samples were randomly assigned to each gel. Protein samples were run on gels following previously described procedures (Thornton et al., 2017). In brief, electrophoresis was performed at 140 V for 90 min at 4°C on 10% polyacrylamide gels using a Bio-Rad Mini PROTEAN tetra cell (Bio-Rad, Hercules, CA) box in running buffer containing 25 mM Tris, 192 mM glycine, and 0.1% (w/v) SDS. The proteins were then transferred to a polyvinylidene difluoride membrane (Bio-Rad) at 100 V for 90 min at 4°C in a transfer buffer consisting of 20% (w/v) methanol, 25 mM Tris, 192 mM glycine, and 0.05% (w/v) SDS. Membranes containing the proteins were then blocked in a 5% (w/ v) nonfat milk and Tris-buffered saline (TBS; 100 mM Tris-HCl, 0.13 M NaCl, and 0.0027 M KCl) solution for 2 h at room temperature. Membranes were then rinsed 2 times in TBS, and then incubated with primary antibodies (HSP\u00ed1, dilution 1:500, product \u00edPA125494, ThermoFisher; HSPA, dilution 1:1,000, product #4872S, Cell Signaling Technologies, Danvers, MA; and DJ1, 1:1,000 dilution, product #ab18257, Abcam, Cambridge, MA) in 5% nonfat milk-TBS solution overnight at 4°C. Membranes were then briefly washed in TBS and incubated in an anti-rabbit secondary antibody (1:1,000 dilution, product #7074S, Cell Signaling Technologies) for 2 h at room temperature. Blots were then briefly washed in TBS and then placed in Western Sure Premium Chemiluminescent Substrate (LI-COR, Lincoln, NE) and imaged using a C-DiGit Blot Scanner (LI-COR). The band intensity of each band was quantified using Image Studio software (LI-COR). The density of the internal standard on each gel for each protein analyzed was used to standardize between different gels by adjusting the value of each sample relative to the internal standard.

Preparation of steaks for meat quality analyses

The same loin used for the previous sample collection (the left strip loin of each carcass) was also used for meat quality analysis. Strip loins were vacuum packaged and allowed to wet age for 14 d postmortem at refrigeration temperatures (4°C). After the aging period, loins were frozen whole at -20°C and stored prior to being fabricated into steaks. Frozen subprimal loins were cut using a band-saw (Butcher Boy; Model #SA-16; American Meat Equipment, LLC, Selmer, TN) into 2.5-cm-thick steaks that were then placed into individual vacuum packaging and stored at -20°C until further analyses were completed. Six consecutively cut steaks from each loin were used for meat quality testing: 1 for shear force, 2 for sensory, and 3 were retained for future needs. The first steak cut was always used for shear force, and the second 2 were always used for sensory to ensure that differences in steak location were not introducing bias into the analysis. Live animal ear tag number, carcass number, and loin number and steak number identification were used to link and identify individual animal to individual steak at each stage of meat production.

Cooking procedures for sensory and Warner-Bratzler shear force (WBSF) analysis are described as follows. Steaks were allowed to thaw for 24 h at 4°C in vacuum packaging. Steaks were trimmed to leave only the LT muscle. Prior to being placed on the grill, an initial internal temperature was recorded for each sample using a thermometer (IPX waterproof thermocouple; 352 Aqua Tuff; Cooper-Atkins, Middlefield, CT). Steaks were placed on a clam shell grill (Griddler Deluxe; Cuisinart; GR-150; East Windsor, NJ) at a grill surface temperature of 232°C and cooked to a medium degree of doneness (internal temperature of 70°C) monitored by an internal thermometer (IPX waterproof thermocouple; Cooper-Atkins).

WBSF

WBSF values were collected from 14-d-aged loin samples (N=99) using methods outlined by the American Meat Science Association (American Meat

Science Association, 2015). Steak preparation and cooking was completed following the methods outlined earlier. After cooking, steaks were covered with plastic wrap on metal trays and allowed to rest 24 h at 4°C. Steaks were then allowed to reach 23°C for a minimum of 1 h before being cored. Seven 1.27 cm core samples were taken from each steak sample following the grain of the longitudinal muscle fibers to be sheared on a TMS-Pro Texture Analyzer (FTC 500N ILC, Food Technology Corporation, Sterling, VA) with a specific blade attachment for WBSF using a 200 mm/min crosshead speed and a 500 kg load cell.

Sensory analysis

For sensory analysis, steaks were cooked as described earlier, and sample preparation followed guidelines developed by the American Meat Science Association (2015). Following cooking, steaks were allowed to rest for 3 min before being cut into one 2×2 cm and three 1×1 cm samples and placed in a plastic sample cup with a plastic lid. The 2×2 cm samples were used to evaluate tenderness and juiciness, while the 1×1 cm samples were used for evaluation

Table 1. Sensory aroma and flavor references and definitions on a 1- to 15-point numerical scale with 0.5 increments and references associated with each attribute that were used to train the panel

Attribute	Definition	Reference associated with attribute		
Beef flavor*	Amount of beef flavor	Swanson's (Campbell Soup Company, Camden, NJ) beef broth = 6.0 80% lean ground beef = 7.0 Beef brisket = 11.0		
Bloody/ serumy*	Aromatics of blood on cooked meat products, closely related to metallic aromatic.	USDA Choice strip steak = 5.5 Beef brisket = 6.0		
Brown/ roasted*	Full aromatic generally associated with broiled beef suet	Beef suet = 8.5 80% lean ground beef = 10.0		
Fat-like*	Aromatics associated with cooked animal fat	Hillshire Farms Lit'l Beef Smokies (Hillshire Farms, Chicago, IL) = 7.0 Beef suet = 12.0		
Liver-like	Aromatics associated with cooked organ meat/liver	Flat iron steak = 3.0 Beef liver = 13.0		
Oxidation	Aromatics associated with aged oil and fat	Beef suet $= 2.0$		
Bitter*	Taste factor associated with caffeine solution	0.01% caffeine solution = 2.0 0.02% caffeine solution = 3.5		
Salty*	Taste factor associated with sodium chloride solution	0.15% sodium chloride solution = 1.5 0.25% sodium chloride solution = 3.5		
Sour*	Taste factor associated with citric acid solution	0.015% citric acid solution = 1.5 0.050% citric acid solution = 3.5		
Umami*	Flat, salty, brothy; taste of glutamate, salts of amino acids and nucleotides	Swanson's beef broth $= 4.5$		
Tenderness	Ease with which sample can be cut through with molars after 3-4 chews			
Juiciness	Amount of juice released during the first 3–4 chews or the amount of moisture removed from the mouth during the first 3–4 chews (dryness)			

^{*}Major attributes: attributes present in 99% of beef samples evaluated by Adhikari et al. (2011) sensory panel.

of beef flavor attributes. Samples were placed on a warm clay brick (preheated in an oven at approximately 121° C) to maintain sample temperature during evaluation. Samples were evaluated under red lighting. Distilled water and unsalted crackers were used as palette cleansers between each sample. Sensory evaluation was conducted at the Utah State University Department of Nutrition, Dietetics, and Food Science facilities by a trained flavor and texture descriptive panel (n = 8) with 12 beef lexicon attributes on a 16-point numerical scale with 0.5 increments (Adhikari et al., 2011). The panelists had over 3 y of experience in sensory evaluation of beef quality attributes. Fifteen training sessions were conducted using a series of steaks ranging in tenderness, juiciness, and beef flavor. Panelists' training used reference anchors outlined by the beef flavor and texture lexicon using previously described methods (Adhikari et al., 2011) to give a 0 to 15 numerical value of intensity for 12 beef sensory attributes, with 0 being slight, 7 the middle point, and 15 strong (Table 1). The panelists evaluated the steaks over a total of 10 different sessions.

Myofibrillar fragmentation index

Skeletal muscle from samples aged for 14 d postmortem were ground under liquid nitrogen, and myofibril fragments were extracted using previously described methods (Culler et al., 1978). In brief, ground tissue was submerged in myofibrillar fragmentation index (MFI) buffer (100 mM KCl, 20 mM KPO₄⁻ [pH 7], 1 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid, 1 mM MgCl₂, and 1 mM NaN₃). Tissue and MFI buffer were homogenized for 30 s. Samples were centrifuged twice at $1,000 \times g$ for 15 min at 4°C; supernatant was decanted, and f resh MFI buffer was added each time after samples were centrifuged. Myofibrillar fragments were then quantified using the Pierce BCA Protein Assay Kit (ThermoFisher Scientific). After quantification, samples were diluted to 500 µg/mL, plated, and read at 540 nm on a BioTek Synergy H1 plate reader (BioTek, Winooski, VT). Myofibrillar fragmentation index was calculated using previously described methods (Culler et al., 1978).

Statistical analysis

The mean, minimum value, maximum value, and standard deviation of WBSF, MFI, carcass characteristics and sensory data of all samples can be seen in Table 2. All analyses were completed using the PROC MIXED procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC). The protein abundance values were continuous independent variables, and the blot and

kill date were treated as random effects. Residuals from this analysis, which are the protein abundance values adjusted for the effects of blot and kill date, were then used as continuous independent variables. The sensory data for each steak were collected from each of the 8 different panelists, and the average of the scores determined by each panelist for each trait were used in the statistical analysis. The sensory-related and carcass characteristics data were analyzed using stepwise linear regression implemented by backward elimination. The sensoryrelated attributes of the meat and carcass characteristics were the independent variables. Upon obtaining a final model for each trait or characteristic, containing kill date and all continuous effects that were deemed important $(P \le 0.10)$, the least-squares means for each sensoryrelated attribute were predicted at the 25th and 75th percentiles of the distribution of any continuous variables remaining in the model. Correlations were determined using Pearson correlations. P < 0.05 was considered statistically significant, whereas P < 0.10 was considered a trend in the data. All data are presented as the leastsquares mean \pm SEM.

Results

The mean, minimum value, maximum value, and standard deviation of the carcass characteristics, loin weights, flavor attributes, and tenderness measurements can be found in Table 2.

Carcass characteristics and loin weights

Protein abundance of HSPA, HSP β 1, or DJ1 in samples collected at day 0 or day 14 was not associated (P > 0.10) with any of the measured carcass characteristics or loin weights (data not shown).

Tenderness

Abundance of HSPA in samples taken at day 0 or after 14 d of aging was not associated (P > 0.10) with tenderness as assessed by WBSF or MFI or with tenderness as determined by a sensory panel (data not shown). Furthermore, abundance of HSP β 1 or DJ1 in samples collected on day 0 was not associated (P > 0.10) with WBSF, MFI, or sensory panel tenderness. In samples collected after 14 d of aging, abundance of DJ1 was found to be a predictor of WBSF (P < 0.001), MFI (P = 0.02), and sensory panel tenderness (P < 0.001) (Table 3). Additionally, abundance of HSP β 1 after 14 d of aging was found to be a predictor of

Table 2. Overview of tenderness and sensory attributes of *longissimus thoracis* samples utilized in the present study

Trait	Mean	Minimum value	Maximum value	Standard deviation
Hot carcass weight, kg	425.1	303.6	508.6	39.2
Loin weight, kg	7.52	5.64	9.32	0.78
Backfat thickness, cm	1.12	0.20	2.24	0.40
Ribeye area, cm ²	94.3	75.5	115.5	7.9
Marbling score	392.1	304.0	575.0	69.2
Yield grade	2.82	1.5	5.0	0.67
WBSF, N	35.1	19.4	54.8	6.9
\mathbf{MFI}^1	42.5	7.5	153.5	29.3
Beef flavor ²	7.29	2.19	8.10	0.64
Bloody/serumy ²	3.36	1.17	5.00	0.65
Brown/roasted ²	6.42	4.83	8.71	0.78
Fat like ²	2.17	1.10	3.75	0.54
Liver like ²	0.52	0.00	1.57	0.38
Oxidized ²	0.69	0.00	2.00	0.40
Sour ²	0.85	0.36	1.75	0.28
Bitter ²	0.53	0.00	1.07	0.21
Salty ²	1.15	0.58	1.88	0.29
Umami ²	2.92	1.50	4.36	0.51
Tenderness ²	7.63	1.76	10.38	1.14
Juiciness ²	7.40	1.76	9.33	1.03

¹MFI was calculated as described by Culler et al (1978).

MFI, myofibrillar fragmentation index; WBSF, Warner-Bratzler shear force.

Table 3. Effects of high and low levels of abundance of DJ1 or HSPβ1 after 14-d aging on WBSF, MFI, or sensory panel tenderness in the *longissimus thoracis*

	Abundance level			
Trait	High ¹	Low ¹	Difference ¹	Probability ²
DJ1				
WBSF, N	35.7 ± 0.59	33.54 ± 0.59	2.16 ± 0.59	< 0.001
MFI^3	50.5 ± 4.1	39.2 ± 4.3	11.4 ± 4.7	0.02
Tenderness ³	7.48 ± 0.12	7.91 ± 0.12	-0.43 ± 0.11	< 0.001
HSPβ1				
MFI ³	37.3 ± 4.9	51.0 ± 4.4	-13.7 ± 6.2	0.03

¹The tabled values are those predicted by the regression at the 25th (low) and 75th (high) percentiles of the distribution of protein abundance levels. Thus, they are nominally equivalent to least-squares means of the low and high groups had the data been divided in high and low classes. It is also important to note that each analysis was completed independently, and as such, the samples that are in the "low" and "high" groups are not the same for each protein or for each trait.

MFI (P = 0.03) but was not associated (P > 0.10) with WBSF or tenderness (Table 3).

Flavor attributes

Using regression analysis, abundance of DJ1 was found to be a predictor of juiciness (P = 0.04) and

tended to predict umami (P = 0.06) in samples of beef collected pre-rigor (Table 4). However, abundance of DJ1 in samples collected on day 0 was not (P > 0.10) a predictor of any of the other sensory attributes listed in Table 1 (data not shown). Furthermore, in samples collected after 14 d of aging, abundance of DJ1

²These values were determined by a trained taste panel as described in Table 1.

 $^{^{2}\}alpha = 0.05$.

 $^{^{3}}$ Tenderness values are those determined by the trained taste panel as described in Table 1. MFI was calculated as described by Culler et al (1978). DJ1, deglycase; HSP β 1, heat shock protein β 1; MFI, myofibrillar fragmentation index; WBSF, Warner-Bratzler shear force.

Table 4. Effects of high and low levels of abundance of DJ1, HSPβ1, or HSPA in samples collected pre-rigor on juiciness, umami, or fat-like flavor in the *longissimus thoracis*

	Abundance level			
Trait	High ¹	Low ¹	Difference ¹	Probability ²
DJ1				_
Juiciness ³	7.38 ± 0.14	7.73 ± 0.14	-0.35 ± 0.16	0.04
Umami ³	2.84 ± 0.06	2.99 ± 0.06	-0.14 ± 0.08	0.06
HSPβ1				
Juiciness ³	3.63 ± 4.9	3.37 ± 4.4	0.25 ± 0.06	< 0.001
HSPA				
Juiciness ³	7.64 ± 0.13	7.45 ± 0.12	0.19 ± 0.11	0.09
Fat-like flavor ³	2.27 ± 0.07	2.19 ± 0.07	0.08 ± 0.05	0.07

¹The tabled values are those predicted by the regression at the 25th (low) and 75th (high) percentiles of the distribution of protein abundance levels. Thus, they are nominally equivalent to least-squares means of the low and high groups had the data been divided in high and low classes. It is also important to note that each analysis was completed independently, and as such, the samples that are in the "low" and "high" groups are not the same for each protein or for each trait.

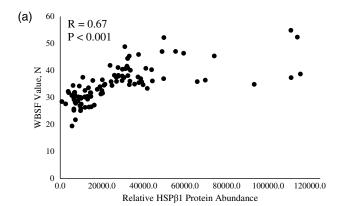
DJ1, deglycase; HSPA, heat shock protein A; HSP β 1, heat shock protein β 1.

was not found to be predictive (P > 0.10) of any of the sensory attributes listed in Table 1 (data not shown). Abundance of HSPB1 after 14 d of aging was also not found to be a predictor (P > 0.10) of any of the sensory attributes shown in Table 1 (data not shown). However, abundance of HSP\(\beta\)1 in samples collected on day 0 was a predictor (P = 0.03) of juiciness (Table 4) but was not a predictor of any of the other sensory attributes shown in Table 1 (data not shown). Regression analysis also demonstrated that abundance of HSPA assessed after 14 d of aging was not a predictor (P > 0.10) of any of the sensory attributes shown in Table 1 (data not shown). However, abundance of HSPA in samples collected at day 0 was a predictor for both juiciness (P = 0.09) and fat-like flavor (P =0.07) but was not associated with any of the other sensory attributes listed in Table 1 (Table 4).

Correlations between sensory attributes, WBSF, MFI, carcass characteristics, and protein abundance of HSP\(\beta\)1, HSPA, and DJ1

The measured abundance of HSP β 1 in samples collected at day 0 and after 14 d of aging was positively correlated with WBSF (P < 0.001) (Figure 1). Additionally, abundance of DJ1 in samples collected at both time points was also positively correlated with WBSF (P < 0.001) (Figure 2). However, abundance of HSPA was not correlated with WBSF (P > 0.05) in samples collected at either time point (data not shown). Sensory analysis of beef flavor, umami, tenderness,

and juiciness were each found to be negatively correlated (P < 0.05) with WBSF (Table 5). Furthermore, abundance of HSP β 1 and DJ1 at both 0 d and 14 d were



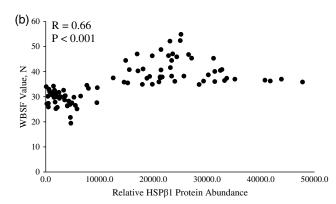
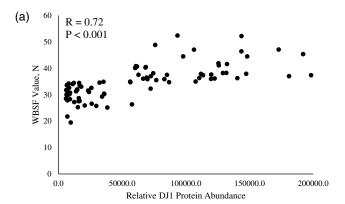


Figure 1. Correlation between protein abundance of heat shock protein beta 1 (HSP β 1) and Warner-Bratzler shear force (WBSF) value (N = 99) of 14-d-aged steaks. Panel A describes the correlation in samples collected pre-rigor, and panel B describes the correlation in samples collected after 14 d of aging. Correlations were conducted using Spearman-Pearson correlations.

 $^{^{2}\}alpha = 0.05$

³These values were determined by a trained taste panel as described in Table 1.



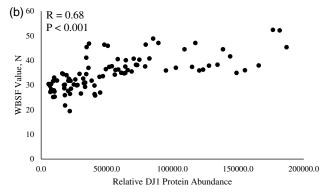


Figure 2. Correlation between protein abundance of a protein involved in oxidative stress (deglycase [DJ1]) and Warner-Bratzler shear force (WBSF) value (N = 99) of 14-d-aged steaks. Panel A describes the correlation in samples collected pre-rigor, and panel B describes the correlation in samples collected after 14 d of aging. Correlations were conducted using Spearman-Pearson correlations.

found to be negatively (P < 0.05) correlated with umami, tenderness, and juiciness (Table 5). Abundance of HSP β 1 at 14 d was also negatively (P < 0.05) correlated with brown/roasted flavor (Table 5). In addition, WBSF was negatively (P < 0.05) correlated with backfat thickness and marbling score and positively (P < 0.05) correlated with yield grade (Table 5). Abundance of HSP β 1 at day 0 or day 14 showed no (P > 0.05) correlation with any of the carcass characteristics (Table 5). However, abundance of HSPA collected at 0 d exhibited a negative (P < 0.05) correlation with loin weight (Table 5). Yield grade was also negatively (P < 0.05) correlated with DJ1 collected at 0 d (Table 5).

Discussion

Skeletal muscle becomes meat through metabolic changes that occur within muscle during the postmortem conversion to meat. Postmortem metabolism creates a situation in which myocytes, the cells that compose the skeletal muscle, undergo cellular stress. A number of physiological process are altered within the stressed myocytes, including a switch from aerobic to anaerobic metabolism, a drop in pH, temperature fluctuations, and an increase in proteolytic enzymes

Table 5. Correlation between protein abundance, sensory values, and MFI

	WBSF ¹	HSPβ1 0 d¹	HSβ1 14 d¹	DJ1 0 d ¹	DJ1 14 d ¹	HSPA 0 d ¹	HSPA 14 d ¹
Beef flavor ²	-0.21*	-0.15	-0.17	-0.1	-0.15	-0.03	0.004
Bloody/serumy ²	-0.09	-0.17	-0.14	-0.09	-0.01	0.11	0.24
Brown/roasted ²	-0.17	-0.13	-0.3*	-0.17	-0.13	-0.04	-0.13
Fat like ²	-0.02	-0.11	-0.14	0.09	0.09	0.06	-0.04
Liver like ²	-0.11	0.04	0.1	0.02	-0.09	-0.11	-0.02
Oxidized ²	0.08	0.1	0.12	0.03	0.11	0.02	-0.15
Sour ²	-0.01	-0.04	0.15	0.07	0.07	0.12	0.11
Bitter ²	0.05	0.03	0.11	0.08	0.09	0.08	0.07
Salty ²	-0.18	-0.06	-0.17	-0.04	-0.08	0.04	-0.01
Umami ²	-0.3*	-0.2*	-0.33*	-0.31*	-0.28*	-0.003	0.06
Tenderness ²	-0.55*	-0.44*	-0.55*	-0.44*	-0.48*	0.02	0.08
Juiciness ²	-0.25*	-0.3*	-0.34*	-0.24*	-0.21*	0.10	0.06
MFI	-0.04	0.07	-0.02	0.10	0.17	-0.02	-0.10
Hot carcass weight	-0.10	-0.12	-0.12	-0.10	-0.13	-0.22	0.02
Loin weight	-0.02	-0.10	-0.006	-0.008	-0.08	-0.26*	-0.02
Backfat thickness	-0.21*	-0.04	-0.01	-0.17	-0.19	-0.17	-0.11
Ribeye area	0.05	0.02	-0.01	0.07	-0.10	-0.13	-0.04
Marbling score	-0.21*	-0.05	-0.10	-0.13	-0.06	-0.16	0.07
Yield grade	0.72*	-0.12	-0.06	-0.21*	-0.15	-0.15	-0.04

¹Values represent the R value between the variables found in corresponding rows and columns; values with a "*" indicate a correlation of $P \le 0.05$.

²Sensory values obtained using a trained panel, based on a 15-point numerical scale outlined in Adhikari et al. (2011).

DJ1, deglycase; HSPA, heat shock protein A; HSP β 1, heat shock protein β 1; MFI, myofibrillar fragmentation index; WBSF, Warner-Bratzler shear force.

(Morzel et al., 2008). The rate and extent of postmortem breakdown of myofibrillar proteins is a major determinant of end-product tenderness (Koohmaraie and Geesink, 2006; Laville et al., 2009). Several muscle endopeptidases, such as calpains and cathepsins, have a major role in the tenderizing process and have been studied at length, although questions remain as to what is responsible for the variability that still remains in these processes (Sentandreu et al., 2002; Pulford et al., 2008). Recent high-throughput analyses have determined that anti-apoptotic proteins, such as HSP and DJ1, may be contributing to variability in tenderness (Guillemin et al., 2011b; Hocquette et al., 2012b; Ma and Kim, 2020). As molecular chaperones, HSP assist with protein assembly, protein folding and unfolding, translocation, interacting with denatured proteins, and preventing protein aggregation to maintain cellular homeostasis and ultimately prevent apoptosis (Xing et al., 2018). After exsanguination, muscle cells become stressed as oxygen supply is depleted. HSP appear as soon as the cell is stressed, and contribute to proper conformation of proteins and preservation of their biological function (Herrera-Mendez et al., 2006). DJ1 is another protein that is thought to prevent cellular apoptosis (Laville et al., 2009; Longo et al., 2015). DJ1 is believed to prevent cellular apoptosis by protecting the cell from oxidative damages (Meulener et al., 2005; McNally et al., 2011). The goal of this research was to determine the relationship between abundance of proteins known to protect the cell from apoptosis (HSPA, HSPβ1, and DJ1) and beef tenderness, sensory traits, and carcass characteristics in order to better understand the factors responsible for meat quality. The present study utilized both linear regression analyses—to determine whether abundance of the proteins of interest could be used to predict the measured meat quality traits—and carcass characteristics, as well as correlations, in order to more loosely determine whether there is a relationship between the proteins of interest and meat quality and carcass characteristics.

The present study evaluated the relationship between carcass characteristics and protein abundance of HSPβ1, HSPA, and DJ1. Breeds of cattle that have a more excitable temperament, such as *Bos indicus* cattle, are more susceptible to stress and often produce a lower-quality carcass compared with *Bos taurus* bred cattle (Crouse et al., 1989; Shackelford et al., 1995; Voisinet et al., 1997; Petherick et al., 2002). Recent research has demonstrated that *Bos indicus* cattle have increased abundance of HSPA and HSPβ1 in skeletal muscle compared with *Bos taurus* cattle (Adamowicz

et al., 2005; Lamb et al., 2007; Mullins et al., 2016). As such, the authors of the present study hypothesized that abundance of HSPA, HSPβ1, and DJ1 would have a negative relationship with desirable carcass characteristics (lower yield grade, increased marbling score, decreased "waste" fat, and increased REA). However, in the present study, regression analyses demonstrated that abundance of neither HSPA, HSPβ1, nor DJ1 measured at either time point was a predictor of any of the measured carcass characteristics. Additionally, abundance of HSPβ1 at either time point showed no correlation with any of the carcass characteristics. HSPA collected pre-rigor was negatively correlated with loin weight, and DJ1 at 0 d was negatively correlated with yield grade; although significant, the R^2 values demonstrate that less than 10% of the variability in these traits is explained by abundance of these proteins. Previous research indicates that steers with increased yield grade exhibit decreased expression of HSP\u00e31 (Keady et al., 2013). Abundance of DJ1 was also previously found to be related with carcass growth (Picard et al., 2017) and quality grade (Thornton et al., 2017). To date, very little research assessing the relationship of HSPA, HSPβ1, and DJ1 with carcass characteristics has been completed. Although the present study found little relationship between abundance of these proteins and carcass characteristics, more research needs to be done to further assess whether these relationships exist in different scenarios, such as when a larger number of animals is assessed, or whether differences exist between breeds.

Many previous studies have found that abundance of both genes and proteins involved in protecting cells from apoptosis, such as HSPA, HSPβ1, and DJ1, have a relationship with development of meat tenderness (Guillemin et al., 2011a; D'Alessandro et al., 2012b; Picard and Gagaoua, 2017; Rosa et al., 2017; Malheiros et al., 2018; Malheiros et al., 2019; Gagaoua et al., 2020; Ma and Kim, 2020). The tenderization process is driven by proteolysis of key myofibrillar proteins in conjunction with other factors such as the amount of lipid and connective tissue present (Koohmaraie, 1994; Koohmaraie et al., 2002; Bekhit et al., 2014; Lana and Zolla, 2016). As such, it has been hypothesized that the presence of proteins known to protect the cell from stress inhibit the proteolysis process, thus resulting in less tender meat (D'Alessandro et al., 2012b; Ouali et al., 2013; Picard and Gagaoua, 2017). HSPA is an HSP that is both constitutively expressed and upregulated in response to stress and is ATP dependent (Welch, 1992; Creagh et al., 2000). Because HSPA is ATP dependent, its role postmortem is not fully understood. HSPB1 is a member of the small HSP family, is ATP independent, and is known to specifically protect several key myofibrillar proteins such as actin, troponin, and tropomyosin, which is why it is the most studied HSP in relationship to meat quality (Morzel et al., 2008; Mymrikov et al., 2011; Balan et al., 2014; Carvalho et al., 2014; Lomiwes et al., 2014; Cassar-Malek et al., 2015). DJ1 is another anti-apoptotic protein that is thought to inhibit the postmortem tenderization by inhibiting proteolysis (Laville et al., 2009; Longo et al., 2015). However, DJ1 prevents apoptosis by protecting the cell from oxidative damage (Meulener et al., 2005; McNally et al., 2011). In the present study, the authors hypothesized that decreased abundance of HSPA, HSPβ1, and DJ1 would be predictors of beef tenderness. In the present study, neither abundance of HSPA nor of HSPβ1 could be used to predict tenderness. However, our results demonstrate that abundance of HSPβ1 in the LT both pre-rigor and at 14 d of aging is correlated with tenderness. These 2 different analyses reveal that there is a relationship between tenderness and abundance of HSP\$1 but do not indicate that HSP\u00e31 is a causative factor in development of tenderness. Additionally, DJ1 protein abundance was found to predict tenderness. Taken together, these results indicate that abundance of both DJ1 and HSPβ1 are correlated with tenderness and DJ1 can be used to predict tenderness, whereas HSPA was not shown to have a relationship with tenderness.

Several previous studies have analyzed the relationship of HSPA, HSPβ1, and DJ1 with tenderness. An association between decreased tenderness and HSPA protein abundance in 2 different muscles of 3 French breeds of cattle was previously identified (Picard et al., 2014). Furthermore, a study utilizing Charolais bulls and steers observed a negative correlation between HSPA abundance and tenderness in the LT (Hocquette et al., 2012a). Similar to HSPA, increased HSP\u00e31 protein abundance in meat with decreased tenderness has been observed (Kim et al., 2008; Balan et al., 2014). Studies involving a mice strain devoid of HSP\u00e31 saw higher myofibril denaturation, and higher intermyofibrillar space, than control mice in postmortem muscle (Cassar-Malek et al., 2015; Picard et al., 2016). In a group of Nellore bulls, researchers observed downregulation of HSP\u00e31 in moderately tender meat compared with very tough meat from the LT (Malheiros et al., 2018). Research involving Italian Simmental bulls showed that a positive correlation existed between HSP\u00e31 and shear force values. In addition, lower HSP\u00e31 protein abundance was observed in the LL when compared with the infraspinatus (Saccà et al., 2015). Furthermore, a previous study associated increased tenderness with DJ1 abundance using principal components analysis in 2 muscles across 3 breeds of cattle (Picard et al., 2013). In Holstein cattle between 29 and 90 mo of age, DJ1 was relatively high in abundance in the semimembranosus (SM) and had the lowest abundance in the semitendinosus (ST); however, the ST and the SM did not differ in tenderness (Ellies-Oury et al., 2019). Samples taken from the LT of Norwegian Red bulls had less DJ1 abundance in more tender samples using two-dimensional gel electrophoresis, but there was no difference in DJ1 abundance between tender and tough samples when analyzed via western blot (Jia et al., 2009). The results of the present study generally agree with the aforementioned studies in that increased abundance of HSPA, HSPβ1, and DJ1 was observed in less tender samples. However, a number of other studies have reported results that conflict with the findings of the present study as well as previously completed studies. HSPA abundance was found to be positively correlated with tenderness in the LT of Charolais bulls (Gagaoua et al., 2018). Similarly, in Norwegian Red heifers and steers, HSPA abundance was positively correlated with tenderness in the SM (Grabež et al., 2015). A recent study also reported no differences of HSP\u00e31 abundance in 12 different skeletal muscles from Aberdeen Angus steers of beef cattle (Temizkan et al., 2019). A positive relationship between tenderness and HSP\(\beta\)1 in the LT and negative relationship in the ST has also been observed (Picard et al., 2014). Furthermore, previous research also indicates increased HSP\(\beta\)1 in more tender samples from the LT in Nellore bulls and steers (Rosa et al., 2018). These conflicting results demonstrate that more research needs to be done to determine the role that HSPA, HSPβ1, and DJ1 may have in development of beef tenderness.

Although a number of studies have analyzed the relationship of HSPA, HSPβ1, and DJ1 with tenderness, few studies have analyzed whether there is a relationship between abundance of these protein and meat quality as assessed by a trained sensory panel. The results of the present study demonstrate that in samples collected pre-rigor, abundance of DJ1 is a predictor of juiciness and umami, abundance of HSPβ1 is a predictor of juiciness, and abundance of HSPβ1 is a predictor of juiciness and fat-like flavor. Marbling score is one of the main factors known to impact juiciness (Platter et al., 2003; Thompson, 2004). Interestingly, there was no observed relationship between HSPβ1,

HSPA, or DJ1 and marbling score, despite the fact each of these proteins was found to predict juiciness. In another study, samples perceived as more tender, juicy, and flavorful by trained sensory panel had decreased abundance of HSP\u00e31 in the LT of Charolais bulls (Bernard et al., 2007). Increased abundance of DJ1 was also observed to be associated with decreased juiciness of ST and LT muscles samples in a study of Saler bulls (Picard et al., 2011). Few studies have evaluated the relationship between anti-apoptotic proteins and beef sensory traits. Results from the present study and others indicate that samples with less HSPA, HSPβ1, and DJ1 are more tender, juicy, and flavorful. Taken together, these results indicate that HSP and DJ1 may be potential biomarkers for beef tenderness; however, more research is needed to fully understand the relationship between abundance of HSP and DJ1 and beef sensory characteristics.

Conclusions

The results of this study demonstrate that abundance of HSPA, HSP\u00e31, and DJ1 have a relationship with both tenderness and meat quality as assessed by a trained sensory panel but do not exhibit any type of relationship with carcass characteristics. The measured abundance of DJ1 in the LT in samples collected after 14 d of aging was found to be a predictor of tenderness. Abundance of HSPβ1 showed a strong correlation with tenderness but was not found to be a predictor of tenderness when analyzed via linear regression. HSPA was not found to be a predictor of tenderness. In addition, in samples collected pre-rigor, abundance of DJ1 is a predictor of juiciness and umami, abundance of HSPβ1 is a predictor of juiciness, and abundance of HSPA is a predictor of juiciness and fat-like flavor. The results of the present study provide important insight into the relationship of abundance of HSPA, HSPβ1, and DJ1 with both meat quality and carcass characteristics. However, additional research is needed to provide more clarity about how these proteins are involved in the development of meat quality in order to produce higher-quality beef.

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