

Sorting for beef tenderness using high performance liquid chromatography and capillary electrophoresis: A research note [☆]

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Abstract

This study utilized two sampling methods to examine changes in sarcoplasmic proteins during aging of beef and their relation to tenderness. Water-soluble proteins either obtained by manually expressing exudates from the meat (*drip*) or by an extraction procedure using homogenization and centrifugation (*ext*) were analyzed for *longissimus lumborum* muscle using HPLC and capillary electrophoresis (CE) on days 2, 7, 10 and 14 postmortem. A peak that consistently increased with aging was identified using HPLC. Among nine peaks detected in the CE analysis, peak 9 (100 kDa) that increased and peak 4 (30 kDa) that decreased with aging were correlated ($P < 0.05$) to tenderness as determined by Warner–Bratzler shear force (WBSF). For pooled data of all aging periods, *drip* sample explained the most variability (49%) in shear force compared to *ext* sample (25%) using HPLC analyses. At 2 days postmortem, a multiple linear regression model explained 83% of the variation in WBSF using CE-*ext* or HPLC-*drip* samples. Sixty percent of the variability in shear force was explained by CE-*ext* samples for day 7 data. The variability in shear force as explained by either *drip* or *ext* sample was less than 51 percent for 10 and 14 days postmortem data. The *drip* samples were comparable to *ext* samples in predicting WBSF values for both tough (>46 N WBSF on day 2) and tender (<46 N WBSF on day 2) strip loins using CE and HPLC procedure. Results suggest that a simple *drip* sampling may have a potential for use with either HPLC or CE analyses on day 2 postmortem for sorting carcasses into tenderness groups.

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Keywords: Tenderness; High performance liquid chromatography; Beef; Aging; Capillary electrophoresis

1. Introduction

The eating quality of beef depends on a number of organoleptic properties including tenderness, appearance, color, intramuscular fat content, taste, and texture. While color and fat content are predominant

traits considered when consumers purchase beef, tenderness is the most important quality characteristic that influences the satisfaction of and decision to repurchase meat (Koohmaraie, Wheeler, & Shackelford, 1995; Morgan et al., 1991). Achieving consistent tenderness in beef has been difficult despite the efforts to standardize breeding, management, nutrition, age and post harvest handling. In order to control the problem of inconsistent tenderness, it is important to have a better understanding of the mechanisms that affect meat tenderness during postmortem aging.

The conversion of muscle to meat and subsequent tenderization typically are a result of the activity of several pH-dependent enzyme systems (Huff-Lonergan

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& Lonergan, 1999). Most research has pointed to the calpain proteolytic enzyme family as being the major factor responsible for the protein degradation that causes meat tenderization (Koochmaraie, 1996). During aging, sarcomeric and cytoskeletal proteins, such as titin, nebulin (Taylor, Geesink, Thompson, Koochmaraie, & Goll, 1995), desmin (Rowe, Maddock, Lonergan, & Huff-Lonergan, 2001) and troponin-T (TnT) (Lonergan, Huff-Lonergan, Wiegand, & Kriese-Anderson, 2001) are degraded.

The identification and quantification of aging-induced proteolytic products offer the potential for predicting beef tenderness. A 110 kDa myofibrillar fragment, found to originate from C-protein (Casserly, Stoeva, Voelter, Healy, & Troy, 1998), appears during the aging of beef (O'Halloran, Troy, & Buckley, 1997). A 30 kDa myofibrillar degradation product of TnT has been reported to be highly correlated to meat tenderness (Koochmaraie, 1994; Muroya et al., 2004; Wheeler & Koochmaraie, 1999). Using high performance liquid chromatography (HPLC) analysis, Stoeva, Byrne, Mullen, Troy, and Voelter (2000) identified three components of trichloroacetic acid (TCA) soluble proteins which increased with aging, while Voelter et al. (2000) employed a number of analytical techniques to separate and sequence a 30 kDa protein fragment identified as a breakdown product of TnT. This led to the development of an ELISA test for predicting tenderness based on the TnT 16–31 fragment obtained from TCA soluble extracts (Tsitsilonis et al., 2002). Kristensen, Therkildsen, and Ertbjerg (2003) employed capillary electrophoresis to identify four protein peaks (P3, P6, P22, P36) from pork muscle early postmortem (1 day), which they reported to be good indicators of tenderness ($R^2 = 0.69$). Lametsch et al. (2003) used proteomic analysis and isolated 19 myofibrillar and 8 sarcoplasmic protein fragments at 72 h postmortem of porcine muscle for their correlations to Warner–Bratzler shear force (WBSF). In their study, three of the actin fragments and the myosin heavy chain fragment were significantly correlated to tenderness. A recent study (Sawdy, Kaiser, St-Pierre, & Wick, 2004) identified seven electrophoretic bands at 36 h postmortem which were significantly correlated to beef tenderness ($R^2 = 0.82$) at 7 days. Mass spectrometric analyses of two of these seven bands were characterized as fragments of bovine myosin heavy chain (bMHC). Their study documented the cellular mechanism in MHC proteolysis during the aging process.

Although isolation of myofibrillar protein fragments has been shown to be useful indicators of proteolysis and tenderness, sample preparation and analyses can be quite lengthy. Voelter et al. (2000) noted the superiority of capillary electrophoresis (CE) to HPLC due to more rapid separations, higher resolution and sensitivity. Kolczak, Pospiech, Palka, and Lacki (2003) investigated changes in myofibrillar and centrifugal drip proteins dur-

ing postmortem aging of *psoas major* and *minor* and *semitendinosus* muscles from calves, heifers and cows. They reported an increase in 30 kDa protein in myofibrillar protein samples during aging whereas, a 26–28 kDa protein decreased with aging in centrifugal drip samples.

Carcasses in large processing plants are broken down into various cuts, vacuum packaged and prepared for distribution within 48 h post slaughter. If there was a sensitive, rapid technique for predicting meat tenderness before carcasses are processed for distribution, it could result in improved marketing opportunities and greatly reduce costs of aging of meat. Ideally, a non- or minimally destructive technique could be employed, and the analysis should be rapid, sensitive and highly correlated to tenderness. The objectives of this study were: (1) to compare two sampling techniques for obtaining protein samples; a manual expression of exudates from the meat (*drip method*), and an extraction procedure using homogenization and centrifugation (*ext method*), (2) to monitor changes of sarcoplasmic proteins during aging of beef strip loins using HPLC and CE analysis, and (3) to determine the relationship of those changes to tenderness measurements as determined by WBSF analysis.

2. Materials and methods

2.1. Meat samples

Ten USDA Select grade strip loins (*longissimus lumborum* muscle) were purchased within 24 h post slaughter from a local processor (J.W. Treuth & Sons, Inc., Catonsville, MD) and stored overnight at 4 °C. On day 2 postmortem, the strip loins were cut into four sections (~6.5 cm each) and randomly assigned to one of four aging periods (2, 7, 10 or 14 days). Samples for 7, 10 and 14 days aging were vacuum packaged and stored at 4 °C. Day 2 samples were cut into two 2.5 cm steaks for WBSF analysis and the interior steak (~1.5 cm) between those two steaks was used for protein analysis. After each aging period, samples ($n = 2$) were cut similarly for WBSF and protein analysis.

2.2. Warner–Bratzler shear force determination

Steaks were cooked on an electric grill (Model GGR50B, Salton, Inc., Mt. Prospect, IL) from an initial temperature of 4–6 °C to an end point temperature of 71 °C following AMSA (1995) guidelines. The temperature was monitored using a type J thermocouple inserted into the geometric center of each steak and connected to a temperature meter (Model HH21, Omega Engineering, Stamford, CT). The steaks were turned once when the internal temperature reached 40 °C. After the cooked steaks cooled to room temperature, a 1.27 cm coring tool was used to remove 10 or more cores parallel to the

direction of the muscle fibers from each steak. Each core was sheared once perpendicular to the muscle fibers with a Warner–Bratzler meat shear blade (1.8 mm thick) mounted on a texture measurement instrument (Model TMS-90, Food Technology Corp., Sterling, VA) at a crosshead speed of 250 mm/min. The peak shear value (N) was recorded for each core, and all values from the same steak were averaged to obtain the mean shear force.

2.3. Extraction of muscle proteins

Samples for protein analysis (1.5 cm thick steaks) were maintained on ice in plastic bags prior to the preparation of the *ext* and *drip* fractions. Each sample was trimmed of visible fat and connective tissue, and starting from the lateral half closest to the dorsal side, a longitudinal strip (~8 g) was removed from the central region. The sliver was placed in between a single layer of cheesecloth, transferred to a sandwich bag, and the *drip* fraction was expressed by manually squeezing the sample through the bag. The *drip* fraction collected at the bottom of the bag was transferred into a 15 ml conical tube and kept on ice for approximately 1 h to allow particulates to settle. The *drip* (~0.3 ml) was diluted 15× with deionized water before further analysis.

The *ext* fraction was prepared according to the method described by Spanier and Romanowski (2000) with few modifications. After removing the muscle sliver for *drip* fraction preparation, the remaining portion of the muscle sample (~60 g) was sliced into pieces using scissors and then minced for 5 s with a handheld blender (Braun MR 370; Braun Inc., Woburn, MA). Five grams of the minced sample were homogenized in a Waring blender with a stainless steel cup using 45 ml of homogenization media [HM consisting of 0.05 M Tris (Tris-HCl) with 1.5 mM dithiothreitol and 1.5 mM tetrasodium ethylenediaminetetraacetic acid (EDTA), pH 7.0]. Homogenization speed was controlled by a rheostat (Powerstat™, Superior Electric Co., Bristol, CT) at a setting of 60 for 10 s. The resulting total homogenate (TH) was filtered through two layers of cheesecloth to remove large particles, residual fat and connective tissue. A 30 ml aliquot of TH was centrifuged at 2800g for 15 min at 4 °C. The pellet was discarded and the supernatant was centrifuged at 48,200g for 45 min at 4 °C. This final supernatant (*ext* sample) was assayed for protein content using method described by Spanier and Romanowski (2000). Both *ext* and *drip* fractions were adjusted to a final concentration of 8 mg/ml using HM and deionized water, respectively.

2.4. High performance liquid chromatography

2.4.1. Sample preparation

Frozen *ext* and *drip* samples (500 µl) were thawed at room temperature for 10 min. A 120 µl aliquot of

thawed *ext* or *drip* samples were diluted to 2 mg/ml protein concentration and mixed well before passing through a centrifuge filter (300 K Omega Nanosep, Pall Life Science, Ann Arbor, MI) that had been prewashed with deionized water. The samples were filter centrifuged for 12 min at 16,000g then the filtrate was transferred to a glass vial.

2.4.2. HPLC conditions

The *ext* and *drip* samples were analyzed by a HPLC system with autosampler (HPLC model, Prostar 410, SD 200 pumps and 200 µl PEEK injector loop, Varian Instrument Co., Walnut Creek, CA). Separations were carried out on a wide pore reverse phase C5 column (5 µm, 250 × 4.6 mm, Supelco, Bellefonte, CA) attached to a C5 guard column (5 µm, 20 × 4 mm, Supelco) using a TFA/CH₃CN gradient system, [Buffer A – 0.1% TFA in water (TFA, Aldrich Chemicals Co., Milwaukee, WI), Buffer B – 0.09% TFA in CH₃CN (EM scientific, Gibbstown, NJ)]. A photodiode array detector (Prostar, Varian Instrument Co., Walnut Creek, CA) was used for the detection at 225, 254 and 280 nm. Run gradients and conditions were: 71% A–29% B at 0.47 ml/min for 19 min, 20% A–80% B at 0.83 ml/min for 18 min and 71% A–29% B at 0.47 ml/min for 9 min. The Star Chromatography Workstation software (version 5.52, Varian) was used to operate the HPLC system and manage data during analysis.

2.5. Capillary electrophoresis

A capillary electrophoresis P/ACE 5510 (Beckman Coulter Inc., Fullerton, CA) controlled by P/ACE station software (version 1.2, Beckman) and SDS coated capillary (100 µm internal diameter, 270 mm length, Beckman) was used. Electrophoresis procedure and sample preparation were according to Beckman Instructions for the SDS 14-200 kit. The kit was comprised of gel buffer, orange G reference marker, sample buffer (0.12 M Tris/HCl, 1% SDS, pH 6.6) and protein standards containing 7 proteins ranging from 14.2 to 205 kDa.

An *ext* or *drip* sample (100 µl), sample buffer (95 µl) and 2-mercaptoethanol (5 µl) were combined in a microcentrifuge tube. The tubes were vortexed then transferred to a boiling water bath for 10 min. The samples were allowed to cool to room temperature, then 25 µl of each aliquot was mixed with 74 µl deionized water and 1 µl Orange G reference marker in a microcentrifuge tube to make 1.0 mg/ml solutions. A 50 µl aliquot of each 1.0 mg/ml solution was transferred to a 200 µl polypropylene vial (Beckman) for CE analysis. Protein standards were analyzed by CE as described by Ngapo and Alexander (1999) for the determination of molecular weight standard curve.

Sample injection was carried out by a 30 s pressure injection. A voltage of 8.1 kV (300 V/cm) and capillary

temperature of 20 °C was used. Peaks were detected at 214 nm. Between runs, the capillary was rinsed with 1 N HCl followed by reconditioning step with gel buffer.

2.6. Data analysis

HPLC, CE and WBSF data were analyzed by two-way ANOVA using a ‘Proc Mixed’ statement (version 8.2, SAS Institute, Inc., Cary, NC) to determine significant differences ($P < 0.05$) due to the aging effect and the sampling procedure. Pearson’s correlation coefficients were calculated between HPLC, CE and WBSF values (version 8.2, SAS Institute, Inc., Cary, NC). Multiple linear regression (MLR) models were developed to predict the WBSF values. The MLR procedure consisted of a stepwise selection search for the best combination of HPLC and CE results in the equation,

$$WBSF_i = \beta_0 + \beta_j X_{ij} + \varepsilon_i,$$

where $WBSF_i$ is the Warner–Bratzler shear force value of the i th sample, $i = 1, \dots, 20$; β_0 is the intercept, β_j is the regression parameter associated with the j th peak, X_{ij} is the relative area for the j th peak of the i th sample, ε_i is the residual error term. The terms in the model (X_{ij}) were added sequentially using the stepwise procedure with a threshold probability of 0.15.

3. Results

3.1. Shear force (WBSF) values

The shear force of beef strip loins varied from 35.40 to 80.95 N on day 2 postmortem. Mean WBSF values decreased over the aging period from 63.41 N on day 2 to 47.40 N at 14 days postmortem, indicating an improvement ($P < 0.05$) in tenderness (Table 1). Beef strip loins were sorted into tough or tender groups based on day 2 WBSF values of >46 and <46 N, respectively. For tender group samples, reduction in WBSF values during the aging period of 14 days postmortem was not different ($P < 0.05$). However, WBSF values of tough samples decreased significantly over 2, 7, and 10 days of aging.

Table 1
Mean and standard deviation for Warner–Bratzler shear force (N) after 2, 7, 10 and 14 days aging

Days	All samples	Tender ($n = 6$) ^A	Tough ($n = 14$) ^A
2	63.41 ^a (± 17.83)	40.60 ^a (± 4.53)	73.18 ^a (± 10.68)
7	54.31 ^b (± 12.20)	39.80 ^a (± 6.71)	60.53 ^b (± 7.86)
10	48.84 ^b (± 10.17)	36.95 ^a (± 6.63)	53.93 ^c (± 6.42)
14	47.40 ^b (± 8.80)	36.30 ^a (± 2.70)	52.15 ^c (± 5.42)

^{a-c} Means with different superscript in the same column are different ($P < 0.05$).

^A Beef strip loins were grouped as Tender (<46 N) and Tough (>46 N) based on day 2 WBSF.

3.2. HPLC profiles

The peak profile of HPLC chromatograms obtained from *ext* or *drip* samples indicated a high absorbing peak (P_B) eluted at 8.7 min. Several peaks were separated after 9 min, however, due to their lower absorption values they were not used in analysis. The peak B (P_B) measured at 225, 254 and 280 nm obtained from *ext* and *drip* samples increased with aging (Table 2). The P_B areas detected at 254 nm from *ext* and *drip* samples increased significantly over each aging period of 2, 7, 10 and 14 days. The P_B areas obtained at 254 nm from *ext* and *drip* samples were strongly correlated ($r = 0.95$, not shown) and similar ($P < 0.05$) at each sampling day. Increase in P_B area of *ext* samples eluted at 225 and 280 nm after 10 and 14 days of aging was significant compared to corresponding peak area after 2 days of aging. The P_B area eluted at 280 nm from *ext* and *drip* samples following 2, 7, 10, and 14 days aging were strongly correlated ($r = 0.77$, not shown).

3.3. CE profiles

Nine peaks were separated using CE for both *ext* and *drip* samples. Peak 4 (30 kDa) and 9 (100 kDa) changed most during aging. The 30 kDa peak decreased, while the 100 kDa peak increased during the 14 days aging period (Table 3). At all aging periods, *drip* samples eluted greater ($P < 0.05$) 30 kDa peak than the 30 kDa peak obtained from *ext* samples. Likewise, the 100 kDa peak obtained from *ext* samples were significantly greater than the 100 kDa peak from *drip* samples on all aging periods. The 30 kDa peak obtained from *ext* or *drip* samples decreased significantly over each aging period of 2, 7, 10, and 14 days.

3.4. Correlation of WBSF to HPLC and CE analyses

Average WBSF values over all aging periods (referred to as pooled data) as well as WBSF values obtained on each sampling day were compared with corresponding

Table 2
Changes in relative area of P_B^A during aging of beef muscle as obtained by HPLC

Days	P_B at 225 nm		P_B at 254 nm		P_B at 280 nm	
	<i>Ext</i>	<i>Drip</i>	<i>Ext</i>	<i>Drip</i>	<i>Ext</i>	<i>Drip</i>
2	6.63 ^{ax}	9.28 ^{ay}	28.25 ^{ax}	24.97 ^{ax}	16.57 ^{ax}	14.45 ^{ax}
7	8.40 ^{abx}	11.56 ^{by}	36.72 ^{bx}	32.08 ^{bx}	19.86 ^{abx}	17.03 ^{ax}
10	10.57 ^{bex}	10.25 ^{abx}	41.05 ^{cx}	36.03 ^{cx}	20.94 ^{bex}	17.22 ^{ay}
14	11.89 ^{cx}	12.22 ^{bx}	46.11 ^{dx}	39.83 ^{dx}	22.85 ^{cx}	17.60 ^{ay}

^{a-d} Values with different superscript in the same column are different ($P < 0.05$).

^{xy} For each peak within sampling procedure, values with different superscript in the same row are different ($P < 0.05$).

^A P_B , relative area of single peak eluted at 8.7 min.

Table 3
Changes in 30 and 100 kDa fragments during aging of beef as identified in electropherogram

Days	30 kDa		100 kDa	
	<i>Ext</i>	<i>Drip</i>	<i>Ext</i>	<i>Drip</i>
2	15.20 ^{ax}	20.18 ^{ay}	2.56 ^{ax}	1.39 ^{ay}
7	11.63 ^{bx}	15.20 ^{by}	4.38 ^{bx}	1.71 ^{aby}
10	8.79 ^{cx}	11.26 ^{cy}	5.06 ^{bx}	2.34 ^{by}
14	6.82 ^{dx}	8.23 ^{dy}	7.38 ^{cx}	3.35 ^{cy}

^{a-d} Values with different superscript in the same column are different ($P < 0.05$).

^{xy} For each peak within sampling procedure, values with different superscript in the same row are different ($P < 0.05$).

peak analyses detected using HPLC and CE procedures. With pooled data, the highest correlation coefficient was found for P_B at 280 nm (-0.57) from *drip* samples and the least for P_B at 225 nm (-0.33) from *ext* samples. The P_B from *ext* and *drip* samples had a negative correlation with WBSF values. Correlation of P_B from *drip* samples was more highly significant to WBSF than the corresponding peak obtained using *ext* samples. When analyzed for each sampling day, P_B at 225 nm (-0.76) from *drip* sample on 2 day postmortem sample was statistically significant. The P_B area detected at 280 nm from *drip* samples was significantly correlated to corresponding WBSF values on all sampling periods except day 7. Most peaks had negative correlation coefficients to WBSF values on 7, 10 and 14 days postmortem.

Strip loins were sorted into tough (>46 N, $n = 14$ steaks) and tender (<46 N, $n = 6$ steaks) groups based on day 2 WBSF values and analyzed for correlation to HPLC. The pooled WBSF values significantly correlated to P_B at 254 and 280 nm obtained using *ext* or *drip* samples from either tender or tough groups. For pooled data, peak analyses from *ext* and *drip* samples were comparable for both, tough and tender group samples.

Postmortem changes identified using CE procedure were also correlated to WBSF values. With pooled data, the 30 kDa fragment obtained from *ext* and *drip* sample showed positive correlation, whereas a 100 kDa fragment from either *ext* or *drip* samples were negatively correlated to WBSF values. When peaks from individual days were compared for their correlation to WBSF values, *ext* samples were superior to *drip* samples. The 30 and 100 kDa fragments from *ext* samples were significantly correlated to WBSF values on 2 and 7 days postmortem.

For pooled data, correlation of CE analysis from *ext* or *drip* samples to WBSF was superior for the tough group samples. A significant correlation with WBSF was found for the 30 kDa fragment obtained in CE analysis for both tender and tough groups. For day 2 values, *ext* samples were more significantly correlated to WBSF than the *drip* samples.

HPLC and CE analyses were used to predict WBSF values using stepwise selection procedure of multiple linear regression models. For pooled data, HPLC method with *drip* sample explained the most variability (49%) in shear force followed by HPLC with *ext* sample (25%), CE method with *drip* sample (19%) and CE with *ext* sample (7%). Furthermore, WBSF values were predicted based on day 2 HPLC and CE analyses. The *drip* method was superior to *ext* method with HPLC explaining 83% of the variability in WBSF. However, *ext* method was superior to *drip* methods using CE explaining 83% of the variability in shear force (Table 4). For 7 days postmortem data, CE method with *ext* sample was far superior explaining 60% of the variability in WBSF. Fifty-three percent of the variability in WBSF was explained by HPLC with *drip* sample for 10 days postmortem samples. Neither HPLC nor CE procedure explained more than 30% of the variability in WBSF for 14 days postmortem samples.

Both *ext* and *drip* samples were comparable in predicting WBSF values of either tough or tender samples. The *drip* from HPLC and *ext* from CE samples explained 77% {WBSF = $8.52 + 0.18 (P_B \text{ at } 254 \text{ nm}) - 0.16 (P_B \text{ at } 225 \text{ nm}) - 0.30 (P_B \text{ at } 260 \text{ nm})$ } and 93% {WBSF = $10.61 + 0.84 (100\text{k peak}) - 0.38 (30\text{k peak})$ } of the variability in shear force of tough groups, respectively, following 2 days postmortem. Using CE, the *drip* samples were superior to *ext* samples in predicting the WBSF values in tender groups; whereas both samples were comparable in WBSF prediction of tender samples when HPLC is employed.

Table 4
Stepwise multiple linear relationship (R^2) between HPLC and CE analyses and Warner–Bratzler shear force (WBSF)

Dependent variable	Days	<i>Drip</i>		<i>Ext</i>	
		HPLC ^a	CE ^b	HPLC ^a	CE ^b
<i>All samples</i>					
WBSF	Pooled data ^c	0.49	0.19	0.25	NS
	2	0.83	0.30	NS	0.83
	7	0.17	NS	0.19	0.60
	10	0.51	NS	NS	NS
	14	0.28	0.28	0.30	NS
<i>Tough samples^d</i>					
WBSF	Pooled data ^c	0.45	0.39	0.30	0.28
	2	0.77	NS	NS	0.93
<i>Tender samples^d</i>					
WBSF	Pooled data ^c	0.40	0.61	0.42	0.39
	2	0.89	0.83	0.93	0.65

NS, no variable met at 0.15 significance level.

^a HPLC includes P_B area measured at 225, 254 and 280 nm, where P_B is the relative area of a peak eluted at 8.7 min.

^b CE includes two peak areas – 30 and 100 kDa.

^c Pooled WBSF values for day 2, 7, 10 and 14.

^d Beef strip loins were grouped as tender (<46 N) and tough (>46 N) based on day 2 WBSF.

4. Discussion

The aim of the present work was to study whether postmortem changes in proteins were related to the shear force values of beef strip loins. We compared two sampling techniques, conventional (*ext*) and simple, much quicker (*drip*) to prepare samples of aged muscle. HPLC and CE procedures were used for their efficacy to analyze postmortem changes using *ext* and *drip* samples. The changes in proteins were compared for correlation with WBSF and potential for predicting shear force values.

The results clearly show that P_B detected by HPLC from *ext* or *drip* samples increases with aging. Collection and preliminary analysis of the P_B eluted at 254 nm using HPLC revealed the presence of at least one ~100 kDa peak (data not shown). CE procedure detected the appearance of two proteins, a 30 kDa peak which decreased with aging and a 100 kDa peak which increased with aging. Proteolysis during the postmortem process of meat has been extensively studied by SDS-PAGE (Geesink & Koohmaraie, 1999; Hwang, Park, Cho, & Lee, 2004; Ilian, Bekhit, & Bickerstaffe, 2004; Ngapo & Alexander, 1999; O'Halloran et al., 1997). O'Halloran et al. (1997) reported the appearance of a 110 kDa fragment in SDS-PAGE profiles of myofibrillar proteins during aging. The origin of the 110 kDa protein, which appears in much smaller quantities than the 30 kDa fragment, was confirmed by Casserly et al. (1998) too. They sequenced this 110 kDa protein and concluded that the solubilization of C-protein caused by the influx of Ca^{2+} ions into the muscle cells postmortem was partly responsible for the weakening of the myofibrils thereby increasing their susceptibility to proteolytic degradation. However, the 100 kDa peak obtained in our CE electropherograms may not necessarily be the same 110 kDa C-protein degradation product, since it appeared in the sarcoplasmic protein fraction. Xiong and Anglemier (1989) reported the gradual appearance of a 100 kDa band in the SDS-PAGE analysis of sarcoplasmic fractions from both ground and intact beef samples and suggested that this 100 kDa protein may originate from α -actinin.

It is widely recognized that the appearance of a 30 kDa band on the SDS-PAGE separation patterns of myofibrils during the postmortem aging of muscle originates from TnT. It has also been demonstrated that this 30 kDa myofibrillar protein increases with aging and is related to postmortem muscle tenderization (Koohmaraie, 1994; Wheeler & Koohmaraie, 1999). The 30 kDa protein in our study, which decreases during aging, seems water-soluble in nature and may not be a myofibrillar degradation product from TnT. Our results are in agreement with Kolczak et al.'s (2003) study which reported a decrease in a 26–28 kDa protein in centrifugal drip with aging. The component(s) represented

by this centrifugal drip fraction have yet to be identified. Similar findings were reported by Nakai, Nishimura, Shimizu, and Arai (1997) who reported a decrease in a 32 kDa sarcoplasmic protein with aging at 4 °C.

While conventional SDS-PAGE techniques are often used in the identification and analyses of proteins in meats, the CE format offers a number of advantages over traditional SDS-PAGE, including a rapid analysis time, on-capillary detection and instrument automation. Ngapo and Alexander (1999) in a comparison of electrophoretic profiles of fresh pork exudates by CE and SDS-PAGE did not detect any differences. Postmortem proteolysis and its relation to pork tenderness were studied by Kristensen et al. (2003) using CE analysis of extractable components after a prerigor freeze-thaw cycle. They reported a significant correlation between WBSF values for 8 of the 39 peaks detected by CE. In our study, significant correlations existed between HPLC and CE analyses and WBSF values. The CE procedure was comparable to HPLC in predicting WBSF values of tough (>46 N WBSF on day 2) and tender (<46 N WBSF on day 2) strip loins. Further, *drip* sampling which is rapid and less tedious than the *ext* sampling was comparable in predicting WBSF values for tough and tender samples using CE and HPLC procedure. It is necessary to predict the tenderness of beef as early as possible to address the need for additional aging or subsequent postmortem treatment. HPLC-*drip* and CE-*ext* procedure explained 77% and 93% of the variability in WBSF of tough groups on day 2, respectively. However, for prescreening tough groups on day 2 postmortem, *ext* sample was superior to *drip* sample in CE procedure and *drip* sample was superior to *ext* sample with HPLC. Both *ext* and *drip* samples were comparable for prescreening fewer tender samples on day 2 postmortem.

Data presented in this study indicate changes in soluble proteins during aging of beef strip loins are related to the tenderness (WBSF values). The relationship is closely associated with 30 and 100 kDa molecular weight proteins. While the source and the identity of these components represented by the two CE peaks and the HPLC peak is yet to be determined, the relative ease of obtaining them in *drip* fractions compared to other proteins of interest in myofibrillar fractions may offer potential for the development of a rapid tenderness prediction method.

5. Conclusion

We report two sampling procedures, a simple and much quicker *drip* and conventional *ext* for obtaining soluble protein samples and subsequent analysis using HPLC and CE to determine protein changes. The protein peaks obtained by CE, the relative area of the peak

eluted in HPLC analysis and their correlation with WBSF to predict tenderness is described. Significant correlation exists between WBSF and these peaks detected by HPLC and CE. A novel *drip* sampling is superior to *ext* sample in predicting tenderness of 2 days postmortem samples using HPLC. However, CE procedure is superior with *ext* samples than the *drip* samples in predicting tenderness of 2 days postmortem samples. Tenderness prediction using components represented by these peaks could be used to identify tender beef samples that do not require additional aging. Continued research on isolation and identification of specific protein(s) of these peaks involved in the postmortem analysis and subsequent development of protein specific antibodies may be useful in developing more reliable and rapid tenderness prediction methods.

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