



Biochemical Factors Affecting East Asian Consumers' Sensory Preferences of Six Beef Shank Cuts

Wan Jun Wu¹, Amelia A. Welter¹, Emily A. Rice¹, Brittany A. Olson¹, Travis G. O'Quinn¹, Elizabeth A. E. Boyle¹, Geraldine Magnin-Bissel², Terry A. Houser³, and Michael D. Chao^{1*}

¹Department of Animal Sciences and Industry, Kansas State University, Manhattan, KS 66506, USA

²Department of Anatomy and Physiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506, USA

³Department of Animal Science, Iowa State University, Ames, IA 50011, USA

*Corresponding author. Email: mdchao@ksu.edu (Michael D. Chao)

Abstract: The objective of this study was to evaluate biochemical factors affecting Warner-Bratzler shear force (WBSF) and East Asian consumers' eating preferences of 6 different beef shank cuts cooked by moist heat. Six different beef shank muscles were collected from 12 USDA Choice beef carcasses ($N = 72$). Shank cuts from the left sides were cooked with moist heat and used for East Asian consumer sensory evaluation and WBSF, and shank cuts from the right sides were left uncooked and used for biochemical analysis and visual panels utilizing the same group of consumers. A correlation analysis was conducted to determine the driving factors that contributed to WBSF and East Asian consumers' overall liking for beef shanks. *Biceps brachii* and *flexor digitorum superficialis-pelvic* received the greatest sensory overall liking, with *deep digital flexor* from the foreshank having the lowest scores ($P < 0.01$). *Deep digital flexor* from the foreshank had the greatest WBSF value, most cooked collagen content, and greatest insoluble collagen percentage as well as the greatest raw and cooked pyridinoline (PYD) densities among all the beef shank cuts ($P < 0.05$). For visual overall liking, shank cuts at approximately 700–750 g such as *biceps brachii* and *extensor carpi radialis* received the highest ratings ($P < 0.01$), and consumers indicated that there was no visual difference in surface color among the shank cuts ($P > 0.10$). Correlation analysis showed that cooked collagen content and insoluble collagen percentage as well as raw PYD densities had positive correlations with WBSF ($P < 0.05$) and negative correlations with consumer overall liking ($P < 0.01$). Surprisingly, collagen content from uncooked shank cuts did not have a direct relationship with consumers' overall liking nor with WBSF. The results demonstrated that raw PYD density may be a great indicator for cooked beef tenderness in beef cuts with a high concentration of connective tissue prepared with moist heat cookery.

Key words: beef shanks, East Asian consumers, connective tissue, tenderness, insoluble collagen, collagen crosslinks

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Introduction

“Beef shank cut” is a general description of a list of locomotive muscles located in beef chuck and round primals that represent approximately 3.3% of the carcass value (Grand View Research, 2019). Beef shank cuts are considered to be low value in the United States due to the high density of connective tissue within the cuts; therefore, the majority of

beef shank meat produced in the US is turned into ground beef (USDA Agricultural Marketing Service, 2020).

Connective tissues will go through a series of conformation changes during cooking depending on the cooking time and temperature (Tornberg, 2005). Martens et al. (1982) reported that the initial collagen conformation change will occur when the cooking temperature reaches between 53°C and

63°C, and this is due to the breakage of hydrogen bonds which results in the contraction of collagen molecules. Furthermore, collagen gelatinization will begin to occur when the temperature reaches 80°C (Palka, 1999; Ismail-Fitry et al., 2011). Jeremiah and Gibson (2003) evaluated different muscles from the round with application of both dry and moist heat cookeries, and they concluded that moist heat cookery was ideal for muscles with a high amount of connective tissue due to the collagen gelatinization effect.

Although beef shank cuts are not well sought after in the US, stewed beef shank is widely consumed in many East Asian cultures, such as China and Taiwan, in the form of “sauces beef” (Mao et al., 2016). The extended cooking time by stewing will soften the connective tissue, and muscle cuts and byproducts containing softened connective tissue are considered a desirable trait to a large portion of Chinese palates (Liu et al., 2017; Xu et al., 2020). This is drastically different from Western culture's perception, whose overall palatability scores tended to have a negative correlation with the total amount of connective tissue in meat (Jeremiah et al., 2003a). Currently, the most popular beef shank cut in Asian markets is the banana shank, which is a group of 3 inseparable muscles (*long digital extensor*, *medial digital extensor*, and *peroneus tertius*) that are located on the most anterior end of the hindshank and ventral to the patella (Jones et al., 2001). It is approximately 900 g (~2 lb) in weight, eye appealing in shape, and most importantly, it contains a high density of connective tissue (Figure 1). Based on a preliminary evaluation of the beef fore- and hindshanks in our laboratory, there are at least 5 other shank cuts that demonstrated similar visual characteristics.



Figure 1. A representative example of banana shank (a combination of *long digital extensor*, *medial digital extensor*, and *peroneus tertius*). The banana shank is characterized by high density of connective tissue as shown in the cross section.

With the extensive economic and population growth for many East Asian countries such as China, South Korea, and Taiwan in recent years, there are opportunities to export the low-value beef shank cuts from beef-producing countries to East Asian countries in order to meet the global demand and bring in additional profit (Mao et al., 2016). However, the eating quality, visual appearance, and biochemical characteristics of beef shanks are not well characterized, and the factors affecting collagen gelatinization in beef shanks are not well understood. Therefore, the objective of this study was to evaluate biochemical factors affecting East Asian consumers' visual and eating preferences of 6 different beef shank cuts.

Materials and Methods

Sample collection and preparation

At approximately 3 d postmortem, bone-in foreshanks (North American Meat Processors Association [NAMP] #117) with the humerus and *biceps brachii* (BB) attached, heel (NAMP #171F) and bone-in hindshanks (NAMP #157) from both sides of 12 USDA Low Choice beef carcasses were collected from a Midwestern beef processor. Following collection, beef cuts were transported to the Kansas State University (KSU) Meat Laboratory (Manhattan, KS), and stored in the cooler overnight at 2°C ± 2°C. Six shank cuts (Figure 2) were fabricated from the subprimals the following day: BB is located dorsal to bone-in foreshank and directly anterior to the humerus. *Triceps brachii* were removed from the humerus at the beef processing plant, leaving only BB and a few minor muscle groups on humerus upon collection. The BB and brachiocephalicus were removed from the humerus, and BB was separated from brachiocephalicus at the natural seam. *Deep digital flexor* from foreshank (DDF-F) is located directly anterior to the radius. The DDF-F and superficial digital flexor-thoracic were removed together as a bundle from the foreshank, and the 2 muscles were separated at their natural seam. The *extensor carpi radialis* (ECR) is located directly posterior to the radius, and it was removed as a single muscle from the foreshank. The *flexor digitorum superficialis-pelvic* (FDS) is located immediately posterior to the ventral end of the femur. The muscle is surrounded by the gastrocnemius and embedded in the center of the heel subprimal. The FDS was removed from the gastrocnemius by carefully separating the muscles at their natural seam. The *deep digital flexor* from hindshank (DDF-H) is located

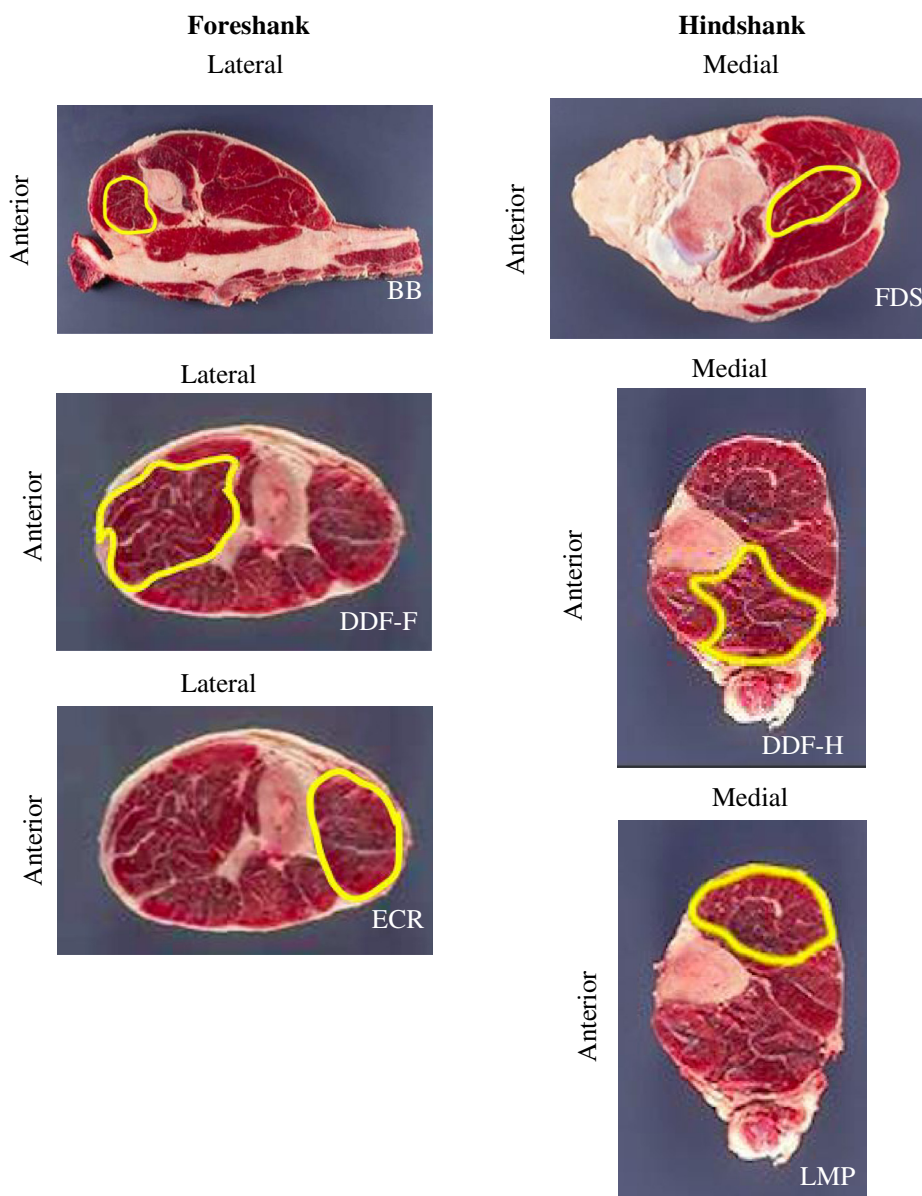


Figure 2. Cross-section of the anatomical location of 6 different beef shank cuts used in this study: *biceps brachii* (BB), *deep digital flexor* from foreshank (DDF-F), *extensor carpi radialis* (ECR), *flexor digitorum superficialis-pelvic* (FDS), *deep digital flexor* from hindshank (DDF-H), and a combination of *long digital extensor*, *medial digital extensor*, and *peroneus tertius* (LMP). Cross section images were downloaded from <http://bovine.unl.edu> (Jones et al., 2001).

immediately lateral to the tibia, and it was removed from the hindshank as a single muscle. Finally, a group of inseparable muscles consisting of the *long digital extensor*, *medial digital extensor*, and *peroneus tertius* (LMP) is located medial to tibia. The 3 muscles were removed from the hindshank and treated as a single cut as this is how this cut is typically sold in Asian markets. Both ends of tendons and accessory muscles were trimmed from all shank cuts.

All 6 cuts from each of the 12 carcasses ($n = 72$) were vacuum packaged and stored in the KSU Meat Laboratory freezer at -40°C . The left sides of the beef

shanks were designated for Warner-Bratzler shear force (WBSF) and East Asian consumer sensory evaluation, and the right sides of the beef shanks were designated for East Asian consumer visual panel, color properties, and biochemical analyses. One of the BB from the right side of the carcass was misplaced and thus excluded from the visual panel and all laboratory analyses ($n = 71$).

Consumer sensory evaluation

East Asian consumers ($n = 91$) were recruited from Manhattan, Kansas, and the surrounding areas.

To ensure the quality of the study, East Asian consumers were selected based on whether they had been exposed to and had a habit of eating beef shanks. Sensory evaluation was conducted on the KSU campus, where consumers were seated in a lecture stadium-style classroom and 6 samples were served in a random order to each consumer. Consumers were supplied with napkins, plastic fork, expectorant cup, unsalted crackers, apple juice, and water to use as palate cleansers between samples. Before evaluation, consumers were given verbal directions to explain the use of palate cleansers, evaluation procedures, and the digital survey.

Whole beef shank muscles from the left side were thawed for 72 h prior to the sensory evaluation at $2^{\circ}\text{C} \pm 2^{\circ}\text{C}$. Prior to cooking, raw weight was taken for each muscle. Each beef shank muscle was stewed for 90 min at $93^{\circ}\text{C} \pm 5^{\circ}\text{C}$ in a half size pan (32 cm \times 27 cm) with 4,000 mL of boiling water using a countertop warmer (X*PRT Series W-3Vi; APW Wyott, Allen, TX). Peak temperature was measured using a probe thermometer at the geometric center of each beef shank cut (Thermapen Mk4; ThermoWorks, American Fork, UT), and cooked beef shank cuts were weighed to obtain cooked weight, which was used in the cooking loss calculation. Beef shank cuts were placed into warming pots and placed on an electric ceramic glass cooktop (GoldSeries; Whirlpool, Benton Harbor, MI) at $50^{\circ}\text{C} \pm 2^{\circ}\text{C}$ to keep warm before serving. Immediately before serving, the dorsal end of each beef shank cut was faced and cut into two 2.5-cm slices. Each slice was cut into 2.5 cm \times 1 cm \times 1 cm cubes, and 2 cubes were served to each consumer. The remaining beef shank of each sample was preserved for WBSF and laboratory analysis.

Each consumer was given an electronic tablet (Model 5709 HP Stream 7; Hewlett-Packard, Palo Alto, CA) with a digital survey (Version 2417833; Qualtrics Software, Provo, UT). Each survey contained a demographic questionnaire and 6 sample ballots in English with Chinese translation. Consumers were asked to evaluate each sample for connective tissue amount, connective tissue texture, tenderness, juiciness, and flavor intensity on Just About Right (JAR) line scales. Anchors were set at 0, 50, and 100, with 0 anchored as too little, too soft, too tough, too dry, and too bland, respectively. A score of 50 was the ideal score, anchored as JAR. At 100, anchors were too much, too hard, too soft, too wet, and too intense, respectively. In addition, consumers were asked to evaluate each sample for overall liking on a 0 to 100 continuous line scale, with 0 anchored as dislike

extremely, 100 anchored as like extremely, and 50 set as the midpoint anchored as neither dislike nor like. Lastly, consumers were also asked to rate each sample as either acceptable or unacceptable with the answer “yes” or “no.” Each consumer evaluated a total of 6 randomized samples with one sample from each beef shank cut.

WBSF

The procedure of WBSF was followed by using the American Meat Science Association Meat Cookery and Sensory Guidelines ([American Meat Science Association, 2015](#)). Two 2.5-cm slices were cut from each cooked beef shank following consumer sensory panel for WBSF evaluation. The cooked beef shank slices were cooled in the cooler at $2^{\circ}\text{C} \pm 2^{\circ}\text{C}$ overnight before the coring and shearing process. Six 1.27-cm-diameter cores that were parallel to the muscle fiber orientation were taken from each cooked beef shank piece and sheared perpendicular to the muscle fiber orientation by using an Instron testing machine (Model 5569; Instron Corporation, Canton, MA) with a cross-head speed of 250 mm/min and a load cell of 100 kg. Measurements were averaged across all 6 cores per sample and recorded as the average peak force (in kilograms).

Consumer visual panel evaluation

Beef shank cuts from the right side of each carcass were thawed 72 h prior to visual panels at $2^{\circ}\text{C} \pm 2^{\circ}\text{C}$. Beef shank cuts were removed from vacuum packages, placed on a Styrofoam tray (#34 and 4S, white; Dyna-Pak, Ontario, Canada) with an absorbent pad, and overwrapped with polyvinyl chloride film (HIYG Gold Stretch Meat film, O_2 transmission rate = 1,191 $\text{cm}^3/0.065 \text{ m}^2/24 \text{ h}$; Berry Global Inc., Evansville, IN). A sticker with the randomized 4-digit number was put on the lower right corner on the wrap for identification purposes. All samples were displayed in coffin-style cases (Model DMF8; Tyler Refrigeration Corporation, Niles, MI) at $2^{\circ}\text{C} \pm 2^{\circ}\text{C}$. The same group of East Asian consumers that had finished the sensory evaluation visually evaluated each beef shank cut under fluorescent lighting (Model F32T8, 32 W, Warm White 3,000 K; Philips Lighting Company, Somerset, NJ) in the KSU Meat Color Laboratory.

Consumers were provided an electronic tablet with a digital survey, and each survey contained 6 sample ballots in English with Chinese translation. The survey program was programmed to assign the order that each beef shank was evaluated. Consumers were asked to

visually evaluate each sample for size and color on JAR line scales. Anchors were set at 0, 50, and 100, with 0 anchored as too small and too light, respectively. A score of 50 was the ideal score, anchored as JAR. At 100, anchors were too large and too dark, respectively. Moreover, consumers were asked to evaluate each sample for overall liking on a 0 to 100 continuous line scale, with 0 anchored as dislike extremely, 100 anchored as like extremely, and 50 set as the midpoint anchored as neither dislike nor like. Lastly, consumers were also asked to rate each sample as either acceptable or unacceptable with the answer “yes” or “no.” Consumers evaluated a total of 6 randomized samples with one sample from each beef shank muscle ($N = 71$). After the visual panels, all beef shank cuts from the right side were vacuum packaged and stored in a -40°C freezer until further analysis.

Color properties

Beef shank cuts from the right side of the carcasses were thawed for 72 h at $2^{\circ}\text{C} \pm 2^{\circ}\text{C}$ prior to secondary fabrication. Beef shank cuts were cut in half in the center, and the dorsal-end halves were overwrapped with polyvinyl chloride film (HIYG Gold Stretch Meat film, Berry Global Inc.) and placed into coffin cases (Model DMF8; Tyler Refrigeration Corporation) at $2^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for at least 30 min prior to color properties measurement. A Hunter Lab MiniScan EZ spectrophotometer (Model 4500L, Illuminant A, 2.54-cm aperture, 10° observer; Hunter Associates Laboratory Inc., Reston, VA) was used to obtain the color measurement on each sample cross section by following the Commission Internationale de l'Éclairage (“International Commission on Illumination”) L^* (lightness), a^* (green to red), and b^* (blue to yellow) system described in the Meat Color Measurement Guidelines (American Meat Science Association, 2012). Color properties were obtained at 6 random locations on the cross section of each sample, and the final value was acquired by averaging the 6 readings. Three 2.54-cm slices were fabricated from the ventral half, vacuumed packaged, and stored at -80°C for laboratory analyses.

Collagen and collagen crosslink sample preparation

Raw and cooked beef shank cuts designated for laboratory analysis were thawed for 24 h prior to pulverization at $2^{\circ}\text{C} \pm 2^{\circ}\text{C}$. Each sample was frozen in liquid nitrogen, pulverized using a commercial-grade blender (Model S1BL32; Waring Products Division, Hartford, CT), transferred into whirl-pak bags (4 oz,

Nasco Inc., Fort Atkinson, WI) and stored in a -80°C freezer until collagen sample preparation.

Collagen sample preparation procedure was based on the protocol described by Avery et al. (2009) with modifications. Briefly, approximately 500 mg of pulverized muscle tissue was weighed into a 15×125 mm glass tube and mixed with 2.5 mL of phosphate-buffered saline containing a 1% sodium borohydride solution (100 mg of sodium borohydride per 1 mL of 0.01 M sodium hydroxide) as a reduction step to prevent the hydrolysis of crosslinks. The samples were incubated for 60 min at room temperature in a fume hood. Approximately 0.2 mL of glacial acetic acid was added into each sample to drop the pH ($\sim\text{pH } 3$) in order to terminate the reduction step. The samples were washed with 3×5 mL of ultrapure water and dried using a Vacuum Evaporation System (RapidVap; Labconco Corporation, Kansas City, MO). After the drying process, 10 mL of 6N hydrochloric acid was added to each sample, and each sample was placed into a drying oven (Isotemp, Fisher Scientific, Hampton, NH) for 24 h at 115°C . After hydrolysis, samples were removed from the oven and evaporated in the Vacuum Evaporation System (RapidVap; Labconco Corporation) until complete dryness (~ 24 h) with the following setting: 70°C , 53% vortex speed, and 200 mbar vacuum. After evaporation, samples were rehydrated with 0.5 mL of ultrapure water and separated into two 250- μL aliquots. The samples were stored at -80°C until analysis.

Collagen content analysis

Both raw and cooked collagen contents were determined by measuring the amount of hydroxyproline described by Bergman and Loxley (1963) with modifications. Hydroxyproline standard curve was prepared on the day of analysis with the following concentrations: 0, 1.5, 3.0, 4.5, 6.0, 7.5, 9.0, and 10.5 $\mu\text{g}/\text{mL}$. Samples were diluted 1:800 with ultrapure water, and 2 mL of diluted sample and standards were mixed with 1 mL of chloramine-T oxidant reagent (6 mM chloramine-T, 140 mM citric acid monohydrate, 38 mM sodium hydroxide, 661 mM sodium acetate trihydrate, and 29% of 1-propanol [$\text{pH } 6$]) and incubated at room temperature for 20 min. After incubation, 1 mL of 4-dimethylaminobenzaldehyde color reagent (67 mM of 4-dimethylaminobenzaldehyde was dissolved in 21% perchloric acid, 35% 2-propanol, and 14% ultrapure water) was added to the samples and standards. Samples and standards were vortexed, covered with aluminum foil, and incubated in water bath for 90 min at

60°C for full color development. Samples and standards were removed from water bath and placed in cold water for 3 min, and 0.2 mL of sample and standards was pipetted to a 96-well plate in duplicate. Hydroxyproline determination was performed using an ultraviolet-visible spectrophotometer (Eon, BioTek Instruments Inc., Winooski, VT) with absorbance set at 558 nm. Sample concentrations were quantified using the known standard curve, and the collagen content was determined by multiplying the hydroxyproline content by 7.14, with a final unit of milligrams of collagen per gram of muscle tissue in dry matter basis. Both raw and cooked collagen content were adjusted to dry matter basis to account for moisture loss during the cooking process. Finally, relative percentages of soluble and insoluble collagen content were calculated from the collagen content measurements from raw and cooked shanks with the following equations:

$$\begin{aligned} \text{Soluble collagen percentage} \\ &= (\text{raw collagen content} - \text{cooked collagen content}) \\ &\div \text{raw collagen content} \end{aligned}$$

$$\begin{aligned} \text{Insoluble collagen percentage} &= \text{cooked collagen content} \\ &\div \text{raw collagen content} \end{aligned}$$

Collagen crosslink analysis

Sample pre-treatment. Mature collagen crosslink determination procedures followed the method described by [Viguet-Carrin et al. \(2009\)](#) with modification. Due to limited resources, only 48 of the 71 meat hydrolysates (8 samples per muscle) previously prepared were used for collagen crosslink analysis. Selected meat hydrolysates were first diluted 1:26 with ultrapure water. Four hundred microliters of the diluted sample and 2.8 mL of sample buffer composed of acetonitrile and glacial acetic acid (6:1 v/v) were mixed, resulting in a total volume of 3.2 mL of prepared sample. Solid Phase Extraction cellulose cartridges (Bond Elut, 300 mg/3 mL; Agilent Technologies, Santa Clara, CA) were first equilibrated with 2.5 mL of wash buffer consisting of acetonitrile, glacial acetic acid, and ultrapure water in 8:1:1 ratio (v/v/v) using a PrepSep 24-Port Vacuum Manifold apparatus (Fisher Scientific, Hampton, NH). Immediately after loading the prepared samples, columns were washed with 4 × 2.5 mL of wash buffer to remove the interfering fluorophores. Care was taken not to let the Solid Phase Extraction column dry out between steps. Finally, crosslinks were eluted with 2 × 0.6 mL of 1% heptafluorobutyric acid (HFBA), and the columns were drained completely. Cleaned samples were transferred

into 2 mL amber vials (*P/N* 5188-6535, Agilent Technologies) capped with a 9-mm pre-slit polytetrafluoroethylene screw cap (*P/N* 5185-5865, Agilent Technologies).

Ultra-performance liquid chromatography conditions

Pyridinoline (PYD) and deoxypyridinoline (DPD) were separated on an ultra-performance liquid chromatography system (Acquity H-Class; Waters Corporation, Milford, MA) equipped with a degasser, a quaternary pump, and a fluorescence detector, and data were processed with the MassLynx chromatography data software (Waters Corp.). Briefly, crosslinks were separated on a reversed phase column (Acquity ultra-performance liquid chromatography HSS T3, 1.8 μm, 2.1 × 100 mm; Waters Corp.) with an injection volume of 5 μL. Flow rate was set at 0.5 mL/min, and the column temperature was maintained at 60°C throughout the run. The chromatographic separation was done using a gradient, with mobile phase A consisting of 0.2% HFBA in ultrapure water and mobile phase B consisting of 100% acetonitrile. PYD and DPD were eluted with 85% solvent A and 15% solvent B at 7.0 and 7.8 min, respectively. After PYD/DPD elution, mobile phase A was decreased to 0% with mobile phase B increased to 100% to elute the hydrophobic residues from the column from 10 to 15 min. At 15.01 min, mobile phase A was changed to 100% until the 20-min mark to equilibrate the column for the next run. The total run time for each sample was 20 min, and PYD and DPD elution was monitored by a fluorescence detector with an emission and excitation wavelength of 395/297 nm. The peak areas were used for PYD and DPD concentration calculations using the linear regression obtained with the standards, and the results were corrected with the dilution factor to obtain final concentration of PYD or DPD. The mature collagen crosslink density was calculated by dividing the molar concentration of PYD and DPD (using a molar mass of 428.44 g/mol and 412.44 g/mol, respectively) by the molar concentration of collagen (using a molar mass of 300,000 g/mol). All mature collagen crosslink density results were expressed as moles of mature collagen crosslink per mole of collagen.

Standard curve and quality control (QC) samples were prepared fresh on the day of the sample analysis using a PYD/DPD high-performance liquid chromatography calibrator (*P/N* 4101, Quidel Corporation, San Diego, CA) diluted with the sample buffer. The standard curve contained a mixture of PYD at 0.02,

0.05, 0.09, 0.19, 0.37, and 0.75 μM and DPD at 0.01, 0.03, 0.05, 0.10, 0.20, and 0.40 μM . A set of QC containing a mixture of PYD at 0.07, 0.33, and 0.65 μM or DPD at 0.04, 0.18, and 0.35 μM was run prior to sample analysis. In addition, QC containing PYD at 0.33 μM and DPD at 0.18 μM was inserted in between every 10 samples to ensure the quality of the analysis. Samples with concentrations out of the standard curve range were further diluted with 0.2% HFBA in 1:5 dilution and re-analyzed.

Moisture and fat analysis

The moisture and fat percentages were determined on the raw samples only using the AOAC 2008.06 method (AOAC, 2012) with a CEM Smart System 5 with Smart Trac Rapid Fat Analysis (CEM Corporation, Matthews, NC). Approximately 3.5 to 4.5 g of pulverized samples was spread evenly in between 2 CEM glass fiber square sample pads (Part #200150; CEM Corporation) and analyzed for moisture percentage using 100% power setting at 125°C. After the moisture analysis, CEM pads containing the samples were placed on top of a Trac film (Part #159875; CEM Corporation), folded and rolled into a cylinder, and fit into the Trac tube (Part #160505; CEM Corporation) for lipid analysis. Both moisture and fat samples were measured in duplicate.

Statistical analyses

Statistical analyses were performed by using the PROC GLIMMIX procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC), and treatment comparisons were considered significant at an α of 0.05. Sensory and visual panels, WBSF, soluble collagen percentage, insoluble collagen percentage, and moisture and fat content were analyzed as a completely randomized block design using a model with the fixed effect of beef shank cuts and each animal as the block. For acceptability data, a model with binomial error distribution was used. The Kenward-Roger approximation was used for all analyses to estimate the degrees of freedom. For collagen content and crosslink (PYD and DPD) analyses, data were analyzed as a split-plot model, with beef shank cuts as the whole-plot factor and cooking treatment as the sub-plot factor of treatment. The Satterthwaite approximation was used to estimate the degrees of freedom. Separation of means was conducted using the LSMEANS procedure with the PDIF option at $P < 0.05$. Finally, the PROC CORR procedure of SAS was used to determine Pearson's correlation coefficients between raw and cooked collagen

characteristics and WBSF and overall liking evaluated by East Asian consumers.

Results

Consumer panel demographics and consumption preference

The demographic profile of the 91 East Asian consumers who participated in the consumer sensory evaluation are presented in Table 1. Participants were primarily Chinese (74.7%), with a similar number of males (50.6%) and females (49.4%). The majority of the participants were married (72.5%), with 56.0% of consumers having a household size of 3–4 people. Moreover, most of the consumers had an annual household income of less than US\$50,000 (63.8%), but with an advanced degree (63.7%). When asked about beef shank meat tenderness preference, 36.3% of consumers preferred their beef shank meat to be cooked to semi-tender (with slight amount of chewiness), and 57.1% of the consumers preferred the beef shank meat to be tender (not chewy but able to maintain structural integrity when shank meat is bitten), with only 6.6% of the consumers preferring that the shank meat fall apart in their mouth. Finally, some consumers ate beef shanks a few times a year (46.2%), followed by every week (28.6%) and every month (24.1%), and 1.1% of consumer indicated that they had never had beef shank meat before.

Consumer sensory evaluation, collagen content and characteristics, and WBSF

Consumer palatability ratings for the 6 beef shank cuts are presented in Table 2. Regarding the amount of connective tissue, BB, FDS, and DDF-H all received similar ratings close to JAR ($P > 0.05$). Consumers rated DDF-F as having too much and ECR and LMP as having too little connective tissue ($P < 0.01$). Among all the beef shank cuts evaluated in this study, East Asian consumers rated DDF-F as having the toughest connective tissue texture ($P < 0.01$), followed by DDF-H, with ECR, LMP, BB, and FDS receiving similar ratings close to JAR ($P > 0.05$). For tenderness rating, BB, FDS, and LMP received similar ratings close to JAR ($P > 0.05$), and ECR and DDF-H were tougher than those rated JAR ($P < 0.01$). DDF-F was the toughest among all for tenderness ($P < 0.01$). Following the same trend as tenderness rating, consumers indicated that BB, FDS, and LMP received similar ratings close to JAR for juiciness ($P > 0.05$), whereas

Table 1. Demographic characteristics of consumers who participated in the East Asian consumer sensory evaluation ($N = 91$)

Characteristic	Response	Percentage of consumers
Sex	Male	50.6
	Female	49.4
Age, y	<20	3.3
	20–29	29.6
	30–39	40.7
	40–49	22.0
	50–59	1.1
	>60	3.3
Ethnicity	Chinese	74.7
	Taiwanese	17.6
	Japanese	2.2
	Other	5.5
Household size	1 person	15.4
	2 people	18.7
	3 people	31.9
	4 people	24.1
	5 people	6.6
	6 people	2.2
	>6 people	1.1
Marital status	Single	27.5
	Married	72.5
Annual household income, US\$	<\$25,000	25.3
	\$25,000–\$34,999	17.6
	\$35,000–\$49,999	20.9
	\$50,000–\$74,999	15.4
	\$75,000–\$99,999	9.8
	\$100,000–\$149,999	5.5
Highest level of education completed	\$150,000–\$199,999	5.5
	High school graduate	11.0
	Some college/technical school	9.9
	College graduate	15.4
Beef shank tenderness preference	Postgraduate	63.7
	Semi-tender	36.3
	Tender	57.1
Beef shank consumption frequency	Falls apart	6.6
	Every week	28.6
	Every month	24.1
	A few times a year	46.2
	Never	1.1

ECR and DDF-H were rated less juicy, and DDF-F was the least juicy among all ($P < 0.01$). A tendency in beef flavor intensity was detected among the 6 beef shank cuts ($P < 0.10$). BB, LMP, and FDS tended to have stronger beef flavor intensity than ECR ($P = 0.06$). Moreover, BB, FDS, and LMP received the highest

sensory overall liking scores, followed by ECR and DDF-H, and DDF-F received the lowest overall liking score among all the shank cuts ($P < 0.01$). As expected, BB, ECR, FDS, DDF-H, and LMP all received high acceptability scores ($>85\%$), and DDF-F was the only one that received less than ideal acceptability scores (62%) compared to the others ($P < 0.01$).

Results for soluble and insoluble collagen percentage and WBSF are shown in Table 3. Collagen content, PYD and DPD density of the 6 different beef shank cuts are shown in Table 4. BB, DDF-H, FDS, and LMP all had the most soluble and least insoluble collagen percentage, followed by ECR, with DDF-F having the least soluble and most insoluble collagen percentage ($P < 0.05$). DDF-F was significantly tougher than the rest of shank cuts when measured by WBSF ($P < 0.01$), and all other beef shank cuts had similar WBSF values ($P > 0.10$).

There was a significant muscle \times cooking treatment interaction for collagen content ($P < 0.01$). In general, DDF-F, FDS, and LMP all had the greatest amount of raw collagen content, followed by BB and DDF-H, with ECR containing the least amount of raw collagen among all the beef shank cuts ($P < 0.01$). However, all the beef shank cuts had similar cooked collagen content except for DDF-F ($P < 0.01$), which had the greatest cooked collagen content among all. In addition, collagen content was reduced after cooking for all the beef shin/shank cuts ($P < 0.01$). There was a significant muscle \times cooking treatment interaction for PYD density. In raw beef shanks, DDF-F had the greatest PYD density, followed by FDS, with BB, ECR, DDF-H, and LMP having the least PYD density ($P < 0.05$). In cooked beef shanks, DDF-F, again, had the greatest PYD density, followed by BB, FDS, and DDF-H, with ECR and LMP having the least PYD density in cooked shanks ($P < 0.05$). There was a cooking effect in which cooking decreased PYD density for DDF-F ($P < 0.05$). Cooking also tended to increase PYD density for DDF-H ($P = 0.05$). However, cooking did not affect PYD density for the rest of the beef shank cuts ($P > 0.10$).

Lastly, there was also a significant muscle \times cooking interaction for DPD density. DDF-F and ECR both had the greatest DPD density in raw beef shanks ($P < 0.01$), followed by FDS, with BB, DDF-H, and LMP having the least DPD density ($P < 0.01$). In cooked beef shanks, BB, ECR, and DDF-H had greater DPD density than DDF-F, FDS, and LMP ($P < 0.01$). There was a cooking effect that showed that cooking decreased DPD density for DDF-F, ECR, and FDS ($P < 0.01$). Cooking did not affect DPD density for the rest of the beef shank cuts ($P > 0.10$).

Table 2. Least-squares means for East Asian consumers' ($N=91$) ratings of palatability traits of 6 different beef shank cuts cooked with moist heat cookery ($93^{\circ}\text{C} \pm 5^{\circ}\text{C}$ for 90 min)

Beef shank	Connective tissue amount ¹	Connective tissue texture ¹	Tenderness ¹	Juiciness ¹	Flavor intensity ¹	Overall liking ²	Acceptability % ³
Foreshank							
BB	47.4 ^c	47.9 ^c	51.0 ^{ab}	49.9 ^a	42.2	69.3 ^{ab}	95.0 ^{ab}
DDF-F	66.1 ^a	75.5 ^a	30.3 ^d	38.3 ^d	38.7	45.6 ^d	62.3 ^c
ECR	39.3 ^d	52.1 ^{bc}	43.4 ^c	43.5 ^{bcd}	34.6	58.9 ^c	88.7 ^b
Hindshank							
FDS	53.3 ^b	45.2 ^c	53.0 ^a	48.8 ^{ab}	39.9	73.1 ^a	97.0 ^a
DDF-H	47.1 ^c	55.9 ^b	45.1 ^{bc}	41.2 ^{cd}	37.7	62.3 ^{bc}	91.9 ^{ab}
LMP	43.8 ^{cd}	51.6 ^{bc}	47.4 ^{abc}	47.3 ^{abc}	40.9	67.8 ^{ab}	93.9 ^{ab}
SEM ⁴	2.35	2.60	2.35	2.31	2.67	3.10	3.19
P value	<0.01	<0.01	<0.01	<0.01	0.06	<0.01	<0.01

¹Sensory evaluation scores: 0 = too little/too soft/too tough/too dry/too bland; 50 = just about right (ideal score); 100 = too much/too hard/too soft/too wet/too intense.

²Sensory evaluation scores: 0 = dislike extremely; 50 = neither like nor dislike; 100 = like extremely.

³Acceptability % = percentage of people who accept the muscle/total number of observations.

⁴SE of the least-squares means.

^{a-d}Least-squares means in a column without a common superscript differ at $P < 0.05$.

BB = biceps brachii; DDF-F = deep digital flexor–foreshank; DDF-H = deep digital flexor–hindshank; ECR = extensor carpi radialis; FDS = flexor digitorum superficialis–pelvic; LMP = long digital extensor, medial digital extensor, and peroneus tertius; SE, standard error.

Table 3. Least-squares means for soluble and insoluble collagen percentage, fat and moisture analysis, cooking loss, and WBSF of 6 different beef shanks ($N=71$)

Beef shank	Soluble collagen, % ¹	Insoluble collagen, % ¹	Fat, %	Moisture, %	Cooking loss, % ²	WBSF, kgf
Foreshank						
BB	45.58 ^{ab}	54.42 ^{bc}	3.6 ^{ab}	73.8 ^{bc}	31.0 ^b	3.3 ^b
DDF-F	27.70 ^c	72.30 ^a	2.9 ^{bc}	74.4 ^b	29.0 ^c	8.9 ^a
ECR	35.95 ^{bc}	64.05 ^{ab}	1.1 ^d	76.3 ^a	33.1 ^a	3.3 ^b
Hindshank						
FDS	57.16 ^a	42.84 ^c	3.9 ^a	73.0 ^d	29.1 ^c	3.9 ^b
DDF-H	43.31 ^{ab}	56.69 ^{bc}	3.2 ^{abc}	73.6 ^{cd}	33.6 ^a	3.6 ^b
LMP	51.94 ^{ab}	48.06 ^{bc}	2.7 ^c	73.7 ^{bc}	27.9 ^c	3.9 ^b
SEM ³	5.38	5.38	0.28	0.31	0.82	2.75
P value	0.01	0.01	<0.01	<0.01	<0.01	<0.01

¹Soluble collagen % = (raw collagen content – cooked collagen content) / raw collagen content. Insoluble collagen % = cooked collagen content / raw collagen content – all in dry matter basis.

²Cooking loss: [(raw weight – cooked weight)/raw weight] × 100.

³SE of the least-squares means.

^{a-d}Least-squares means in a column without a common superscript differ ($P < 0.05$).

BB = biceps brachii; DDF-F = deep digital flexor–foreshank; DDF-H = deep digital flexor–hindshank; ECR = extensor carpi radialis; FDS = flexor digitorum superficialis–pelvic; LMP = long digital extensor, medial digital extensor, and peroneus tertius; SE = standard error; WBSF = Warner-Bratzler shear force.

Fat and moisture percentage and cook loss

Fat and moisture percentage from raw beef shanks and cook loss results are also presented in Table 3. The results from the current study showed that BB, DDF-F, FDS, DDF-H, and LMP all had more fat percentage compared to ECR ($P < 0.01$). As expected, moisture

percentage had an inverse relationship with the fat percentage measurement. The results showed that ECR had the greatest moisture percentage, followed by BB, DDF-F, and LMP, with FDS and DDF-H having the least moisture percentage out of all ($P < 0.01$). Finally, ECR and DDF-H had the greatest cooking loss

Table 4. Least-squares means of raw and cooked collagen content ($n = 71$), PYD ($n = 48$), and DPD ($n = 48$) densities of 6 different beef shanks

Beef shank	Collagen content, mg/g (DM ¹)		PYD density, mol/mol collagen				DPD density, mol/mol collagen					
	Raw	Cooked	SEM ²	<i>P</i> value	Raw	Cooked	SEM ²	<i>P</i> value	Raw	Cooked	SEM ²	<i>P</i> value
Foreshank			4.21	<0.01			0.04	<0.05			0.001	<0.01
BB	57.47 ^{bx}	30.77 ^{by}			0.14 ^{cx}	0.23 ^{bx}			0.008 ^{cx}	0.012 ^{ax}		
DDF-F	65.44 ^{abx}	47.06 ^{ay}			0.54 ^{ax}	0.42 ^{ay}			0.016 ^{ax}	0.008 ^{by}		
ECR	42.23 ^{cx}	27.02 ^{by}			0.19 ^{cx}	0.14 ^{cx}			0.019 ^{ax}	0.013 ^{ay}		
Hindshank												
FDS	75.78 ^{ax}	32.31 ^{by}			0.34 ^{bx}	0.28 ^{bx}			0.014 ^{bx}	0.007 ^{by}		
DDF-H	57.14 ^{bx}	31.19 ^{by}			0.19 ^{cx}	0.31 ^{bx}			0.010 ^{cx}	0.010 ^{ax}		
LMP	75.77 ^{ax}	35.51 ^{by}			0.13 ^{cx}	0.12 ^{cx}			0.010 ^{cx}	0.007 ^{bx}		

¹Raw and cooked collagen content were adjusted to DM basis to account for moisture loss during the cooking process.

²SE of the least-squares means.

^{a-c}Least-squares means in a column without a common superscript differ ($P < 0.05$).

^{x,y}Least-squares means in a row without a common superscript differ ($P < 0.05$).

BB = biceps brachii; DDF-F = deep digital flexor–foreshank; DDF-H = deep digital flexor–hindshank; DM = dry matter; DPD = deoxypyridinoline; ECR = extensor carpi radialis; FDS = flexor digitorum superficialis–pelvic; LMP = long digital extensor, medial digital extensor, and peroneus tertius; PYD = pyridinoline; SE = standard error.

Table 5. Least-squares means for East Asian consumers' ($N = 84$) visual evaluation ratings of 6 different beef shank cuts (uncooked)

Beef shank	Raw weight (g)	Size ¹	Color ¹	Overall liking ²	Acceptability % ³
Foreshank					
BB	724.3 ^b	52.5 ^c	54.2	63.8 ^{ab}	95.4 ^a
DDF-F	881.2 ^a	67.5 ^a	59.3	58.7 ^{bc}	84.8 ^b
ECR	881.5 ^a	59.9 ^b	55.8	67.5 ^a	96.5 ^a
Hindshank					
FDS	435.2 ^c	32.1 ^d	55.7	53.0 ^c	74.1 ^b
DDF-H	936.1 ^a	68.5 ^a	53.3	59.1 ^{bc}	84.8 ^b
LMP	864.8 ^a	67.4 ^a	51.0	59.2 ^{bc}	84.8 ^b
SEM⁴	35.43	2.00	2.51	3.06	3.58
<i>P</i> value	<0.01	<0.01	0.21	0.02	<0.01

¹Visual evaluation scores: 0 = too small/too light; 50 = just about right (ideal score); 100 = too large/too dark.

²Visual evaluation scores: 0 = dislike extremely; 50 = neither like nor dislike; 100 = like extremely.

³Acceptability % = percentage of people who accept the muscle/total number of observations.

⁴SE of the least-squares means.

^{a-d}Least-squares means in a column without a common superscript differ ($P < 0.05$).

BB = biceps brachii; DDF-F = deep digital flexor–foreshank; DDF-H = deep digital flexor–hindshank; ECR = extensor carpi radialis; FDS = flexor digitorum superficialis–pelvic; LMP = long digital extensor, medial digital extensor, and peroneus tertius; SE = standard error.

percentage, followed by BB, and DDF-F, FDS, and LMP had the least cooking loss percentage among the 6 beef shank cuts ($P < 0.01$).

Consumer visual evaluation ratings and color properties

Beef shank raw weight and visual evaluation ratings are presented in Table 5. The results showed that

DDF-F, ECR, DDF-H, and LMP had the greatest raw weight, followed by BB, whereas FDS was the lightest among all the beef shanks ($P < 0.01$). On the other hand, East Asian consumers indicated that BB was close to the JAR beef shank size. East Asian consumers also rated ECR as slightly too big in size and rated DDF-F, DDF-H, and LMP as too big in size ($P < 0.01$). Finally, East Asian consumers rated FDS as too small ($P < 0.01$). East Asian consumers indicated that there

was no difference in surface color for all beef shanks ($P > 0.10$). East Asian consumers also rated BB and ECR with the highest visual overall liking scores, followed by DDF-F, DDF-H, and LMP, with FDS receiving the lowest score ($P < 0.05$). Following the same trend as overall liking, BB and ECR were most visually acceptable ($>95\%$), whereas DDF-F, FDS, DDF-H, and LMP were less acceptable than BB and ECR ($<85\%$; $P < 0.01$).

Beef shank color properties of the cross sections are presented in Table 6. For color properties, FDS had the highest L^* value, followed by BB, DDF-F, ECR, and DDF-H, with LMP having the lowest L^* value ($P < 0.01$). There were no differences found in both a^* and b^* among different beef shank cuts ($P > 0.10$).

Correlations of collagen content, characteristics, and fat and moisture content with WBSF and East Asian consumer sensory overall liking

Presented in Table 7 are correlation coefficients of raw and cooked collagen content, soluble and insoluble collagen percentage, collagen crosslink densities, and fat and moisture percentage with WBSF and overall liking response from the East Asian consumer sensory evaluation of the 6 different beef shanks. WBSF and consumer overall liking showed no significant correlation with raw collagen content ($P > 0.10$). However, cooked collagen content had a strong positive correlation with WBSF ($r = 0.615$; $P < 0.01$) and negative correlation with consumer overall liking ($r = -0.454$; $P < 0.01$). As expected, soluble collagen percentage had a negative correlation with WBSF ($r = -0.392$; $P < 0.05$) and a strong positive correlation with consumer overall liking ($r = 0.612$; $P < 0.01$), and insoluble collagen percentage had a positive correlation with WBSF ($r = 0.392$; $P < 0.05$) and negative correlation with consumer overall liking ($r = -0.612$; $P < 0.01$). Raw PYD density had a strong positive relationship with WBSF ($r = 0.730$; $P < 0.01$) and negative relationship with consumer overall liking ($r = -0.484$; $P < 0.01$). There was still a minor positive correlation between cooked PYD density and WBSF ($r = 0.324$; $P < 0.10$), but the positive relationship was not as strong as for the raw PYD density. Raw DPD density tended to have a positive correlation with WBSF ($r = 0.321$; $P < 0.10$), but there was no significant correlation between cooked DPD density and WBSF or consumer overall liking ($P > 0.10$). Lastly, fat percentage had a minor positive correlation with consumer overall

Table 6. Least-squares means for color properties of 6 different beef shanks ($N = 71$; uncooked)

Beef shank	L^* ¹	a^* ²	b^* ³
Foreshank			
BB	45.5 ^b	24.4	16.1
DDF-F	45.9 ^b	24.5	16.5
ECR	45.6 ^b	25.3	16.2
Hindshank			
FDS	47.7 ^a	25.6	17.3
DDF-H	45.8 ^b	24.1	16.3
LMP	43.4 ^c	23.8	15.9
SEM ⁴	0.65	0.83	0.56
P value	<0.01	0.54	0.47

¹ L^* = lightness (0 = black and 100 = white).

² a^* = redness (−60 = green and 60 = red).

³ b^* = blueness (−60 = blue and 60 = yellow).

⁴SE of the least-squares means.

^{a-c}Least-squares means in a column without a common superscript differ ($P < 0.05$).

BB = biceps brachii; DDF-F = deep digital flexor–foreshank; DDF-H = deep digital flexor–hindshank; ECR = extensor carpi radialis; FDS = flexor digitorum superficialis–pelvic; LMP = long digital extensor, medial digital extensor, and peroneus tertius; SE = standard error.

Table 7. Correlation coefficient (r) of raw and cooked collagen content (DM basis), soluble and insoluble collagen percentage, raw and cooked mature collagen crosslink densities, moisture, and percentage with WBSF and East Asian consumer overall liking of 6 beef shanks

Collagen components	WBSF	Overall liking
Raw collagen content (DM¹)	0.211	0.231
Cooked collagen content (DM¹)	0.615 ^{***}	−0.454 ^{***}
Soluble collagen %	−0.392 ^{**}	0.612 ^{***}
Insoluble collagen %	0.392 ^{**}	−0.612 ^{***}
Raw PYD² density	0.730 ^{***}	−0.484 ^{***}
Cooked PYD² density	0.324 [*]	−0.195
Raw DPD³ density	0.321 [*]	−0.144
Cooked DPD³ density	−0.220	0.192
Raw moisture %	−0.001	−0.272 [*]
Raw lipid %	0.026	0.242 [*]

^{*} $P < 0.10$.

^{**} $P < 0.05$.

^{***} $P < 0.01$.

¹Raw and cooked collagen content were adjusted to DM basis to account for moisture loss during the cooking process.

²Pyridinoline.

³Deoxypyridinoline.

DM = dry matter; DPD = deoxypyridinoline; PYD = pyridinoline; WBSF = Warner-Bratzler shear force.

liking, whereas moisture showed the opposite trend ($P < 0.10$). However, WBSF showed no significant correlation with fat or moisture percentage ($P > 0.10$).

Discussion

In East Asian cultures, tenderness is considered as part of the overall texture (Sasaki et al., 2014). In this study, we specifically measured the sensory attributes using the JAR scale because overly tender may not have a positive relationship with overall liking in East Asian culture. As indicated by the consumer preference survey, only 6.6% of the panelists preferred the fall-apart-in-mouth texture. Therefore, differences between East Asian and the typical US consumer's perceptions of beef tenderness should be taken into consideration when evaluating the results from this study.

Besides being rated as having the highest amount of connective tissue, DDF-F was also rated as having the toughest connective tissue texture and was rated as being the least tender, least juicy, and the least preferred beef shank cut. All the beef shank cuts used in this study were cooked with the same moist heat cookery, cooking time, and temperature, and DDF-F was not the largest of the beef shank cuts (Table 5). Therefore, it is clearly the case that when the connective tissues are not fully gelatinized, an excessive amount of connective tissue in meat may also make it difficult for the consumers to chew and have a direct effect on overall liking. On the other hand, too little connective tissue may not be able to provide the hypothesized gel-lubrication effect, which may also be detrimental to the overall liking of beef shanks as the shanks contain very little fat. This may also explain why the East Asian consumers rated ECR with lower scores for overall liking compared to the other beef shank cuts. Although this concept has been long known within the East Asian community, it is the first time, to the authors' knowledge, that it has been documented in a scientific study. This study also demonstrated that beef shank cuts that had the JAR amount of connective tissue, such as FDS, were the ones that provided the best eating experience for consumers, which also ended with the highest overall liking scores from East Asian consumers. In many past studies, the amount of connective tissue in meat has been shown to have a negative relationship with consumer evaluation of tenderness and overall liking scores (Berry et al., 1988; Jeremiah et al., 2003b; Lorenzen et al., 2003). However, it is important to point out the uniqueness of this study as it specifically focused on East

Asian consumers, and the shank cuts were cooked through moist heat cookery. As mentioned earlier, Asian consumers tend to consider having connective tissue in stewed meat a desirable trait (Mao et al., 2016; Xu et al., 2020). Finally, it is important to note that WBSF between 3.3 and 3.9 kgf is where Asian consumers consider JAR for beef shank tenderness.

Past research has demonstrated that locomotive muscles tended to contain higher collagen content and mature collagen crosslinks but less collagen solubility in comparison to the supportive muscles (Wheeler et al., 2000; Torrescano et al., 2003; Chun et al., 2020). Although the current study only utilized beef shank muscles (all considered locomotive muscles), there were still significant differences in the function and the force-generation capacity of each of the individual muscles/muscle groups used in this study. Both Swanstrom et al. (2004) and Blunden et al. (2006) demonstrated that the forelimb, especially the muscle DDF-F and its tendon, endure a greater amount of force during galloping in racehorses compared to the other locomotive muscles. Furthermore, Brown et al. (2003) studied the force generating capacities from different muscles in horses and reported that DDF muscles had a peak isometric force of 9,504 N whereas ECR had a peak isometric force of 536 N. Because DDF has greater force-generation capacity compared to ECR, DDF was expected to show higher collagen content and greater density of the heat-stable mature collagen crosslinks compared with muscles with less force-generating capacity.

In addition, Torrescano et al. (2003) investigated the insoluble collagen content and WBSF in bovine muscles and reported that muscles with greater insoluble collagen content also tended to have greater WBSF values. The mechanism behind this phenomenon was explained by Steinhart et al. (1994) and Yamauchi et al. (1988), who both reported that the mature crosslinks tended to increase the mechanical and thermal stability of collagen fibers and their tensile strength, which would have a negative effect on collagen solubility. These heat-stable collagen fibers are known to contribute to the texture of connective tissue and meat toughness (Tanzer, 1973). Therefore, it is likely that DDF-F exhibiting the toughest connective tissue texture, the highest WBSF value, and the lowest ratings for tenderness, juiciness, overall liking, and acceptability score were due to its greater proportion of insoluble collagen percentage and greater density in mature collagen crosslinks compared to the other beef shank cuts used in this study.

Although the collagen molecules were made up of α -chains bound by hydrogen bonds in a triple helix

structure making its structure extremely stable, heat treatment is able to break the hydrogen bonds to allow for the solubilization of collagen fibers (Weston et al., 2002). Therefore, we expected to observe collagen loss for the beef shank cuts after cooking because collagen solubilization is expected to occur when the temperature reaches 80°C (Palka, 1999; Ismail-Fitry et al., 2011). In the current study, all the beef shanks were stewed in water at 93°C for 90 min. With such cooking conditions, any collagen that can be easily solubilized should be released, leaving only the “heat-insoluble” collagen behind.

PYD and DPD are major mature collagen crosslinks. PYD is mainly found in muscles (Nakano et al., 1985; Young et al., 1994; Bosselmann et al., 1995), and DPD is mainly found in bones (Robins et al., 1994; Lietz et al., 1997). Raw PYD density may vary due to differences in species, diets, and muscle's intended functionality (Roy et al., 2015). Previous studies demonstrated that, as PYD density increased, collagen solubility decreased in bovine muscles (Smith and Judge, 1991; Bosselmann et al., 1995). Listrat et al. (2007) measured PYD concentration in raw and cooked bovine muscles and reported that the cooked meat expressed higher PYD concentration (nanomoles per milligram of muscle tissue). It is important to note that when the PYD is expressed as concentration (based on weight of the muscle tissue), moisture loss during the cooking process needs to be accounted for. PYD was retained during the cooking process and thus its concentration in the meat was increased. Therefore, the discrepancy between the results of this study and those from Listrat et al. (2007) was strictly because of differences in unit expression. Our findings confirmed that PYD is fairly heat stable, and the network of connective tissue may require extensive heat treatment to solubilize in the presence of a greater density of PYD in beef. Although cooking did not seem to affect PYD density for the majority of the beef shank cuts used in this study, a cooking effect was found for DDF-F. This phenomenon demonstrated that moist heat cookery with extended cooking period could still have an effect on PYD density, particularly meat cuts with inherent high PYD density. However, the exact relationship between PYD density and heat treatment, as well as the potential influence of other collagen crosslinks, is still unknown.

It was expected that DPD density from all the beef shank cuts would be significantly lower than the PYD density. DPD is the crosslink that predominates in bone and is known to be a minor component in connective tissue (Robins et al., 1994; Lietz et al., 1997;

Cremers et al., 2008). Bosselmann et al. (1995) reported that bovine *extensor carpi ulnaris* contained about 0.010 mol/mol of collagen for DPD density, and that value was similar to the DPD density found in beef shanks for this current study. Yoshida et al. (2014) investigated the mature DPD crosslink and its relationship to mechanical properties in mouse cervical tissues and reported that DPD was positively correlated with mechanical properties such as ultimate stiffness, which demonstrated that DPD may be important to tissue tensile strength in certain tissues. Furthermore, Yoshida et al. (2014) also reported that dihydroxylysinonorleucine (DHLNL) is an immature crosslink that may later be converted into the mature DPD crosslink, and they found a strong positive relationship between DHLNL and tissue stiffness. Perhaps the immature collagen crosslink DHLNL may play a role in bovine connective tissue texture, and further studies are needed to elucidate its relationship with beef tenderness.

Kerth and Miller (2015) discussed that the Millard reaction may be the main contributor of beef flavor intensity for cooked beef using dry heat cookery. Since this study utilized moist heat cookery, the interaction between amino acids and reducing sugar was limited. On the other hand, O'Quinn et al. (2012) found that steaks that had greater beef flavor intensity also had greater fat content as lipid degradation products are main contributors to beef flavor. Therefore, the tendency for beef flavor intensity differences found among the beef shank cuts was likely due to fat content differences. The low fat percentage indicated that beef shank cuts are healthy alternatives because of their lower content of fat. According to the USDA definition, beef that contains less than 10 g of fat in 100 g of beef is considered to be “lean,” whereas beef that contains less than 5 g of fat within 100 g of beef is considered to be “extra lean” (USDA Food Safety and Inspection Service, 2014). Therefore, all of the beef shanks from this study met the USDA definition of “extra lean” beef. Font-i-Furnols and Guerrero (2014) reported that global consumers' preferences for beef have changed over time, and consumers preferred leaner beef nowadays for health reasons. Zhang et al. (2017) further reported that this preference for lean meat trend can also be applied in many Asian communities.

Typically, lean meat contains approximately 75% water (Pearce et al., 2011). During the cooking process, meat will lose weight due to protein denaturation and loss of ability to hold water (Purslow et al., 2016). In this study, all the beef shank cuts ended with a peak internal temperature of ~91°C, which was close to the

water temperature in the cooking devices due to the extensive stewing time (90 min). [Olson et al. \(2019\)](#) reported that the top sirloin steaks had ~21% cooking loss in different USDA quality grades utilizing dry heat cookery. In the current study, beef shanks' cooking loss ranged from 28% to 34%. Previous studies had demonstrated that higher cooking losses were sustained when moist heat cookery was utilized, which is in agreement with results from the current study ([Moore et al., 1980](#); [Jeremiah and Gibson, 2003](#)).

Moreover, [Jeremiah et al. \(2003a\)](#) investigated cooking loss and moisture content of different bovine muscles and reported that muscles with higher moisture content would also have higher cooking loss. In the current study, all the beef shank cuts with the exception of DDF-F followed the trend as described by [Jeremiah et al. \(2003a\)](#). [Ismail et al. \(2019\)](#) measured soluble collagen and cooking loss of bovine *semitendinosus* muscle and reported that soluble collagen had a positive relationship with cooking loss. Perhaps the cooking loss discrepancy found for DDF-F was related to its lower collagen solubility as found in this study, which indicates that DDF-F contained collagen that is more resistant to heat-induced gelatinization, resulting in less cooking loss as well as lower scores in tenderness and juiciness.

Evaluation of size was included in the current study because beef shanks are usually sold as a whole muscle cut in East Asian countries and domestic Asian markets, and knowing the ideal beef shank size could help us identify East Asian consumers' purchasing preferences. Based on these results, East Asian consumers preferred a shank size of ~700–750 g. There are 2 explanations for East Asian consumers' preference for medium-sized beef shanks. First, [Nam et al. \(2010\)](#) reported that grains and vegetables are the major components of many East Asian diets, whereas meat consumption is limited. This is very different from Western culture, as [Sweeter et al. \(2005\)](#) showed that US consumers tended to have greater demand for larger pieces of meat such as ribeye steaks. Second, the demographic survey from this study showed that most East Asian consumers have 3–4 people in their household, and the “medium”-size beef shank seem to be the ideal size for a smaller family size.

[Goñi et al. \(2008\)](#) compared instrument-based measurement and visual evaluation with the use of reference standards in beef color measurement, and they reported that the L^* value measured by colorimeter was closely correlated with the visual color evaluation scores. However, the current study showed a discrepancy between the objective and subjective

color measurement. For this study, the epimysium, also referred to as the “silver skin,” was not removed from the surface of the beef shanks because this is how Asian grocery stores typically present these cuts. However, this might have affected East Asian consumers' ability to accurately determine the surface meat color of the beef shanks due to the presence of this silver skin. On the other hand, color property measurement was conducted using the cross sections of beef shanks, for which the silver skin was not in place to influence meat color evaluation. Based on the East Asian consumers' visual evaluation results, overall liking and acceptability were mostly determined by the beef shank size and weight but not the color of the meat.

Much of the past research has reported similar results as found in this current study, which demonstrated no or a mild relationship of raw collagen content with WBSF and consumer eating preferences ([Crouse et al., 1985](#); [DeVol et al., 1988](#); [LaRoche et al., 2020](#)). In the current study, both DDF-F and FDS had similar collagen content; however, the overall liking scores for these 2 muscles were in great contrast to each other. East Asian consumers rated FDS as the most overall liked beef shank among all tested, whereas DDF-F was rated to be the least overall liked among all tested. These results indicated that collagen content in raw meat cuts is not a good indicator of East Asian consumer overall liking for meat cuts cooked with moist heat cookery.

[Torrescano et al. \(2003\)](#) evaluated collagen content and WBSF of “raw” bovine muscles and demonstrated that there was a positive correlation between WBSF and collagen content in raw beef. These results demonstrated that background toughness in raw meat is driven by the total collagen content as the raw collagen still retains its full tensile strength. On the other hand, background toughness in cooked meat is driven by the remaining “cooked” collagen that could not be solubilized during the cooking process. In agreement with the current study, [Riley et al. \(2005\)](#) reported that the insoluble or cooked collagen content had a positive correlation with WBSF in bovine *longissimus dorsi* muscle, and [Jeremiah et al. \(2003b\)](#) found that insoluble collagen content was closely related to the palatability attributes from the sensory panel. An inverse relationship of collagen solubility with WBSF and consumer overall liking was expected. As collagen solubility increased, more collagen could be readily solubilized in meat, resulting in softer connective tissue texture during cooking, which would also decrease in WBSF and improve consumer overall liking of the product.

Many previous studies have demonstrated that the presence of mature collagen crosslinks is related to meat toughness (Bailey and Light, 1989; Lepetit, 2007) or tissue tensile strength (Yoshida et al., 2014). In the current study, raw PYD density had a strong positive correlation with WBSF and consumer overall liking. Mature collagen crosslinks can retain their collagen structure by linking themselves with the neighboring collagen fiber, which provides structure and strength for the collagen and, at the same time, decreases collagen solubility and prevents softening of the connective tissue texture during cooking (Weston et al., 2002; Maynes, 1987). On the other hand, Dubost et al. (2013) and Chun et al. (2020) did not find a relationship between PYD density and meat tenderness in a variety of bovine muscles. Two factors can be used to explain this discrepancy. First, dry heat cookery with short cooking time aiming for an internal temperature of 55°C and 71°C, respectively, was applied for both studies. Dry heat cookery with a short cooking time may not be enough to fully solubilize all the soluble collagen, and so the soluble collagen continued to contribute to meat toughness, thus masking PYD's relationship to meat toughness. Second, most of the beef muscles evaluated in both studies were supportive muscles, which contain very little connective tissue, collagen content, and collagen crosslinks. Therefore, it was difficult to find correlation between collagen crosslinks and meat toughness in supportive muscles because the collagen contribution to meat toughness in those muscles is likely minute compared to the beef shanks utilized in this study.

Cooked PYD density showed no relationship with consumer overall liking and had a positive correlation with WBSF, but not as strong as the raw PYD density. This could be related to the cooking effect found in DDF-F for PYD density. PYD is known to be a heat-stable mature crosslink, and yet the PYD density decreased in DDF-F from raw to cooked shank, which demonstrates that moist heat cookery with extended cooking time could potentially release additional PYD. However, to the best of the authors' knowledge, this is the first known attempt to specifically investigate the effect of extended moist heat cookery on mature crosslink densities. Additional studies are needed to further confirm this observed phenomenon. Moreover, there was a tendency for positive correlation between raw DPD density and WBSF in this study. Yoshida et al. (2014) also reported that DPD was positively correlated with stiffness and mechanical strength in the cervical tissue from mouse. However, DPD's contribution to meat tenderness is still largely unknown within the meat science community.

It is well established that fat percentage is positively correlated with consumer overall liking (Corbin et al., 2015; Drey et al., 2019) because fat can increase the perception of juiciness and potentially reduce the toughness contributed by connective tissue (Thompson, 2001; Nishimura, 2010). However, we did not expect to find such relationship in this particular study because all the shank cuts had less 4% fat, which is lower than the fat percentage found in USDA Select top sirloin steaks (5%) (Olson et al., 2019).

Conclusions

This study filled a knowledge gap and shed light on East Asian consumers' visual and eating preferences of beef shank cuts. The results indicated that tenderness and juiciness of cooked beef shank affected Asian consumers' eating preference of beef shank cuts, whereas shank size was the main factor affecting their visual preference. In addition, cooked (insoluble) collagen is what contributed to the background toughness, and PYD is a heat-stable collagen crosslink that may require extensive heat treatment to degrade and allow for the solubilization of collagen. As a result, raw PYD density may be a good indicator for cooked collagen content and cooked beef tenderness in beef cuts with a high concentration of connective tissue prepared with moist heat cookery. Future studies are needed to investigate the specific relationship among collagen crosslinks, cooking time, and temperature to create an equation that can consistently predict the palatability of the beef cuts cooked with moist heat cookery.

Literature Cited

- American Meat Science Association. 2012. Meat color measurement guidelines. American Meat Science Association, Champaign, IL.
- American Meat Science Association. 2015. Research guidelines for cookery, sensory evaluation, and instrumental tenderness measurements of meat. 2nd ed. American Meat Science Association, Champaign, IL.
- AOAC. 2012. Official Method 2008.06. Official methods of analysis of AOAC International. 19th ed. AOAC International, Gaithersburg, MD.
- Avery, N. C., T. J. Sims, and A. J. Bailey. 2009. Quantitative determination of collagen cross-links. In: Extracellular matrix protocols. Humana Press, Totowa, NJ. p. 103–121.
- Bailey, A., and N. Light. 1989. Connective tissue in meat and meat products. 19th ed. Elsevier Applied Science, London. p. 195–224.

- Bergman, I., and R. Loxley. 1963. Two improved and simplified methods for the spectrophotometric determination of hydroxyproline. *Anal. Chem.* 35:1961–1965. <https://doi.org/10.1021/ac60205a053>.
- Berry, B., J. Smith, J. Secrist, and L. Douglass. 1988. Consumer response to restructured beef steaks processed to have varying levels of connective tissue. *J. Food Quality.* 11:15–25. <https://doi.org/10.1111/j.1745-4557.1988.tb00861.x>.
- Blunden, A., S. Dyson, R. Murray, and M. Schramme. 2006. Histopathology in horses with chronic palmar foot pain and age-matched controls. Part 2: The deep digital flexor tendon. *Equine Vet. J.* 38:23–27. <https://doi.org/10.2746/042516406775374342>.
- Bosselmann, A., C. Möller, H. Steinhart, M. Kirchgessner, and F. J. Schwarz. 1995. Pyridinoline cross-links in bovine muscle collagen. *J. Food Sci.* 60:953–958. <https://doi.org/10.1111/j.1365-2621.1995.tb06269.x>.
- Brown, N. A., M. G. Pandey, C. E. Kawcak, and C. W. McIlwraith. 2003. Force-and moment-generating capacities of muscles in the distal forelimb of the horse. *J. Anat.* 203:101–113. <https://doi.org/10.1046/j.1469-7580.2003.00206.x>.
- Chun, C. K. Y., W. Wu, A. A. Welter, T. G. O'Quinn, G. Magnin-Bissel, D. L. Boyle, and M. D. Chao. 2020. A preliminary investigation of the contribution of different tenderness factors to beef loin, tri-tip and heel tenderness. *Meat Sci.* 170:108247. <https://doi.org/10.1016/j.meatsci.2020.108247>.
- Corbin, C. H., T. G. O'Quinn, A. J. Garmyn, J. F. Legako, M. R. Hunt, T. T. N. Dinh, R. J. Rathmann, J. C. Brooks, and M. F. Miller. 2015. Sensory evaluation of tender beef strip loin steaks of varying marbling levels and quality treatments. *Meat Sci.* 100:24–31. <https://doi.org/10.1016/j.meatsci.2014.09.009>.
- Cremers, S., P. Garnero, and M. Seibel. 2008. Chapter 87—Biochemical markers of bone metabolism. In: *Principles of Bone Biology*. San Diego, California, Academic Press.
- Crouse, J. D., H. R. Cross, and S. C. Seideman. 1985. Effects of sex condition, genotype, diet and carcass electrical stimulation on the collagen content and palatability of two bovine muscles. *J. Anim. Sci.* 60:1228–1234. <https://doi.org/10.2527/jas1985.6051228x>.
- DeVol, D. L., F. K. McKeith, P. J. Bechtel, J. Novakofski, R. D. Shanks, and T. R. Carr. 1988. Variation in composition and palatability traits and relationships between muscle characteristics and palatability in a random sample of pork carcasses. *J. Anim. Sci.* 66:385–395. <https://doi.org/10.2527/jas1988.662385x>.
- Drey, L. N., L. L. Prill, B. A. Olson, E. A. Rice, J. M. Gonzalez, J. L. Vipham, T. A. Houser, E. A. E. Boyle, and T. G. O'Quinn. 2019. Evaluation of marbling and enhancement's abilities to compensate for reduced beef palatability at elevated degrees of doneness. *J. Anim. Sci.* 97:669–686. <https://doi.org/10.1093/jas/sky435>.
- Dubost, A., D. Micol, B. Picard, C. Lethias, D. Andueza, D. Bauchart, and A. Listrat. 2013. Structural and biochemical characteristics of bovine intramuscular connective tissue and beef quality. *Meat Sci.* 95:555–561. <https://doi.org/10.1016/j.meatsci.2013.05.040>.
- Font-i-Furnols, M., and L. Guerrero. 2014. Consumer preference, behavior and perception about meat and meat products: An overview. *Meat Sci.* 98:361–371. <https://doi.org/10.1016/j.meatsci.2014.06.025>.
- Goñi, V., G. Indurain, B. Hernandez, and M. Beriain. 2008. Measuring muscle color in beef using an instrumental method versus visual color scales. *J. Muscle Foods.* 19:209–221. <https://doi.org/10.1111/j.1745-4573.2008.00106.x>.
- Grand View Research. 2019. Beef market size, share & trends analysis report by cut (brisket, shank, loin), by slaughter method (kosher, halal), by region (North America, Europe, APAC, MEA, CSA), and segment forecasts, 2019–2025. <https://www.grandviewresearch.com/industry-analysis/beef-market-analysis>. (Accessed 1 June 2020).
- Ismail, I., Y.-H. Hwang, A. Bakhsh, and S.-T. Joo. 2019. The alternative approach of low temperature-long time cooking on bovine semitendinosus meat quality. *Asian Austral. J. Anim.* 32:282. <https://doi.org/10.5713/ajas.18.0347>.
- Ismail-Fitry, M., A. H. Paterson, B. H. Wilkinson, and R. W. Purchas. 2011. Initial work on developing a cooking protocol for producing re-structured meat under controlled conditions. *Proc. Food Sci.* 1:662–666. <https://doi.org/10.1016/j.profoo.2011.09.099>.
- Jeremiah, L., and L. Gibson. 2003. Cooking influences on the palatability of roasts from the beef hip. *Food Res. Int.* 36:1–9. [https://doi.org/10.1016/S0963-9969\(02\)00093-5](https://doi.org/10.1016/S0963-9969(02)00093-5).
- Jeremiah, L., M. Dugan, J. Aalhus, and L. Gibson. 2003a. Assessment of the relationship between chemical components and palatability of major beef muscles and muscle groups. *Meat Sci.* 65:1013–1019. [https://doi.org/10.1016/S0309-1740\(02\)00309-1](https://doi.org/10.1016/S0309-1740(02)00309-1).
- Jeremiah, L., L. Gibson, J. Aalhus, and M. Dugan. 2003b. Assessment of palatability attributes of the major beef muscles. *Meat Sci.* 65:949–958. [https://doi.org/10.1016/S0309-1740\(02\)00307-8](https://doi.org/10.1016/S0309-1740(02)00307-8).
- Jones, S., D. Burson, and C. Calkins. 2001. Muscle profiling and bovine myology. National Cattlemen's Beef Association CD-Rom, Washington, DC.
- Kerth, C. R., and R. K. Miller. 2015. Beef flavor: A review from chemistry to consumer. *J. Sci. Food Agr.* 95:2783–2798. <https://doi.org/10.1002/jsfa.7204>.
- LaRoche, E. M., W. Wu, C. K. Y. Chun, A. A. Welter, C. K. Jones, A. Crane, T. A. Houser, T. G. O'Quinn, and M. D. Chao. 2020. An investigation of biochemical factors affecting Asian consumers preference for stewed goat meat. International Congress of Meat Science and Technology and American Meat Science Association Reciprocal Meat Conference [Virtual], 2 August 2020 (Abstract). https://meatscience.org/docs/default-source/default-document-library/all-abstracts-in-numerical-order.pdf?sfvrsn=46332612_0. (Accessed 5 January 2021).
- Lepetit, J. 2007. A theoretical approach of the relationships between collagen content, collagen cross-links and meat tenderness. *Meat Sci.* 76:147–159. <https://doi.org/10.1016/j.meatsci.2006.10.027>.
- Lietz, G., A. Avenell, and S. P. Robins. 1997. Short-term effects of dietary sodium intake on bone metabolism in postmenopausal women measured using urinary deoxypyridinoline excretion. *Brit. J. Nutr.* 78:73–82. <https://doi.org/10.1079/bjn19970120>.
- Listrat, A., A. Levieux, and J. Lepetit. 2007. Muscle, age and sex effects on total pyridinoline cross-links content and beef

- tenderness. In 53rd International Congress Meat Science and Technology, Beijing, China. p. 247–248.
- Liu, R., L. Xing, G. Zhou, and W. Zhang. 2017. What is meat in China? *Animal Frontiers*. 7:53–56. <https://doi.org/10.2527/af.2017.0445>.
- Lorenzen, C., R. Miller, J. Taylor, T. Neely, J. Tatum, J. Wise, M. Buyck, J. Reagan, and J. Savell. 2003. Beef customer satisfaction: Trained sensory panel ratings and Warner-Bratzler shear force values. *J. Anim. Sci.* 81:143–149. <https://doi.org/10.2527/2003.811143x>.
- Mao, Y., D. L. Hopkins, Y. Zhang, and X. Luo. 2016. Consumption patterns and consumer attitudes to beef and sheep meat in China. *J. Food Nutr. Res.* 4:30–39. <https://doi.org/10.12691/ajfn-4-2-1>.
- Martens, H., E. Stabursvik, and M. Martens. 1982. Texture and colour changes in meat during cooking related to thermal denaturation of muscle proteins. *J. Texture Stud.* 13: 291–309.
- Maynes, R. 1987. Biology of extracellular matrix: A series. In: R. Mayne, and R. E. Burgeson, editors, *Structure and function of collagen types*. Academic Press, Inc., Orlando, FL.
- Moore, L. J., D. L. Harrison, and A. Dayton. 1980. Differences among top round steaks cooked by dry or moist heat in a conventional or a microwave oven. *J. Food Sci.* 45:777–781. <https://doi.org/10.1111/j.1365-2621.1980.tb07447.x>.
- Nakano, T., J. Thompson, and F. Aherne. 1985. Concentration of the collagen crosslink pyridinoline in porcine skeletal muscle epimysium. *Can. Inst. F. Sci. Tec. J.* 18:100–102. [https://doi.org/10.1016/S0315-5463\(85\)71729-4](https://doi.org/10.1016/S0315-5463(85)71729-4).
- Nam, K.-C., C. Jo, and M. Lee. 2010. Meat products and consumption culture in the East. *Meat Sci.* 86:95–102. <https://doi.org/10.1016/j.meatsci.2010.04.026>.
- Nishimura, T. 2010. The role of intramuscular connective tissue in meat texture. *Anim. Sci. J.* 81:21–27. <https://doi.org/10.1111/j.1740-0929.2009.00696.x>.
- O'Quinn, T., J. Brooks, R. Polkinghorne, A. Garmyn, B. Johnson, J. Starkey, R. Rathmann, and M. Miller. 2012. Consumer assessment of beef strip loin steaks of varying fat levels. *J. Anim. Sci.* 90:626–634. <https://doi.org/10.2527/jas.2011-4282>.
- Olson, B. A., E. A. Rice, L. L. Prill, L. N. Drey, J. M. Gonzalez, J. L. Vipham, M. D. Chao, and T. G. O'Quinn. 2019. Evaluation of beef top sirloin steaks of four quality grades cooked to three degrees of doneness 1. *Meat Muscle Biol.* 3:399–410. <https://doi.org/10.22175/mmb2019.07.0022>.
- Palka, K. 1999. Changes in intramuscular connective tissue and collagen solubility of bovine m. semitendinosus during retorting. *Meat Sci.* 53:189–194. [https://doi.org/10.1016/s0309-1740\(99\)00047-9](https://doi.org/10.1016/s0309-1740(99)00047-9).
- Pearce, K. L., K. Rosenvold, H. J. Andersen, and D. L. Hopkins. 2011. Water distribution and mobility in meat during the conversion of muscle to meat and ageing and the impacts on fresh meat quality attributes—A review. *Meat Sci.* 89:111–124. <https://doi.org/10.1016/j.meatsci.2011.04.007>.
- Purslow, P., S. Oiseth, J. Hughes, and R. Warner. 2016. The structural basis of cooking loss in beef: Variations with temperature and ageing. *Food Res. Int.* 89:739–748. <https://doi.org/10.1016/j.foodres.2016.09.010>.
- Riley, D., D. Johnson, C. Chase Jr., R. West, S. Coleman, T. Olson, and A. Hammond. 2005. Factors influencing tenderness in steaks from Brahman cattle. *Meat Sci.* 70:347–356. <https://doi.org/10.1016/j.meatsci.2005.01.022>.
- Robins, S. P., H. Woitge, R. Hesley, J. Ju, S. Seyedin, and M. J. Seibel. 1994. Direct, enzyme-linked immunoassay for urinary deoxypyridinoline as a specific marker for measuring bone resorption. *J. Bone Miner. Res.* 9:1643–1649. <https://doi.org/10.1002/jbmr.5650091019>.
- Roy, B., J. Aalhus, J. Basarab, I. Larsen, and H. Bruce. 2015. Relationship between meat quality and collagen crosslink concentrations in beef from calf-fed, yearling-fed and mature cattle. *Meat Sci.* 99:142. <https://doi.org/10.1016/j.meatsci.2014.07.017>.
- Sasaki, K., M. Motoyama, T. Narita, T. Hagi, K. Ojima, M. Oe, I. Nakajima, K. Kitsunai, Y. Saito, and H. Hatori. 2014. Characterization and classification of Japanese consumer perceptions for beef tenderness using descriptive texture characteristics assessed by a trained sensory panel. *Meat Sci.* 96:994–1002. <https://doi.org/10.1016/j.meatsci.2013.10.021>.
- Smith, S., and M. Judge. 1991. Relationship between pyridinoline concentration and thermal stability of bovine intramuscular collagen. *J. Anim. Sci.* 69:1989–1993. <https://doi.org/10.2527/1991.6951989x>.
- Steinhart, H., A. Bosselmann, and C. Möller. 1994. Determination of pyridinolines in bovine collagenous tissues. *J. Agr. Food Chem.* 42:1943–1947.
- Swanstrom, M. D., S. M. Stover, M. Hubbard, and D. A. Hawkins. 2004. Determination of passive mechanical properties of the superficial and deep digital flexor muscle-ligament-tendon complexes in the forelimbs of horses. *Am. J. Vet. Res.* 65:188–197. <https://doi.org/10.2460/ajvr.2004.65.188>.
- Sweeter, K., D. Wulf, and R. Maddock. 2005. Determining the optimum beef longissimus muscle size for retail consumers. *J. Anim. Sci.* 83:2598–2604. <https://doi.org/10.2527/2005.83112598x>.
- Tanzer, M. L. 1973. Cross-linking of collagen. *Science*. 180:561–566. <https://doi.org/10.1126/science.180.4086.561>.
- Thompson, J. 2001. The relationship between marbling and sensory traits. Beef CRC Marbling Symposium, Coff's Harbour, Australia, 1 October 2001. p. 30–35. <http://livestocklibrary.com.au/handle/1234/20176>.
- Tornberg, E. 2005. Effects of heat on meat proteins—Implications on structure and quality of meat products. *Meat Sci.* 70:493–508. <https://doi.org/10.1016/j.meatsci.2004.11.021>.
- Torrescano, G., A. Sanchez-Escalante, B. Gimenez, P. Roncales, and J. A. Beltrán. 2003. Shear values of raw samples of 14 bovine muscles and their relation to muscle collagen characteristics. *Meat Sci.* 64:85–91. [https://doi.org/10.1016/S0309-1740\(02\)00165-1](https://doi.org/10.1016/S0309-1740(02)00165-1).
- USDA Agricultural Marketing Service. 2020. National daily cow and boneless beef summary. Livestock, poultry, and grain market news. <https://www.ams.usda.gov/mnreports/lscdbbs.pdf>. (Accessed 1 June 2020).
- USDA Food Safety and Inspection Service. 2014. Beef from farm to table. Food Safety Information. https://www.fsis.usda.gov/wps/wcm/connect/c33b69fe-7041-4f50-9dd0-d098f11d1f13/Beef_from_Farm_to_Table.pdf?MOD=AJPERES#:~:text=%20

- [Here%20are%20their%20definitions%3A,than%2095%20milligrams%20of%20cholesterol.&text=Beef%20is%20aged%20to%20develop%20additional%20tenderness%20and%20flavor.](#) (Accessed 1 June 2020).
- Viguet-Carrin, S., E. Gineyts, C. Bertholon, and P. D. Delmas. 2009. Simple and sensitive method for quantification of fluorescent enzymatic mature and senescent crosslinks of collagen in bone hydrolysate using single-column high performance liquid chromatography. *J. Chromatogr. B.* 877:1–7. <https://doi.org/10.1016/j.jchromb.2008.10.043>.
- Weston, A., R. Rogers, and T. Althen. 2002. The role of collagen in meat tenderness. *Professional Animal Science.* 18:107–111. [https://doi.org/10.15232/S1080-7446\(15\)31497-2](https://doi.org/10.15232/S1080-7446(15)31497-2).
- Wheeler, T. L., S. D. Shackelford, and M. Koohmaraie. 2000. Variation in proteolysis, sarcomere length, collagen content, and tenderness among major pork muscles. *J. Anim Sci.* 78:958–965. <https://doi.org/10.2527/2000.784958x>.
- Xu, C., Y. Wang, J. He, D. Pan, H. Wang, G. Zhou, and J. Cao. 2020. The comparative research of structural and textural characteristics of six kinds of collagen-based sauce braised meat products. *J. Food Sci.* 85:1675–1680. <https://doi.org/10.1111/1750-3841.15119>.
- Yamauchi, M., D. T. Woodley, and G. L. Mechanic. 1988. Aging and cross-linking of skin collagen. *Biochem. Biophys. Res. Co.* 152:898–903. doi:10.1016/s0006-291x(88)80124-4.
- Yoshida, K., H. Jiang, M. Kim, J. Vink, S. Cremers, D. Paik, R. Wapner, M. Mahendroo, and K. Myers. 2014. Quantitative evaluation of collagen crosslinks and corresponding tensile mechanical properties in mouse cervical tissue during normal pregnancy. *PLoS One.* 9:e112391. <https://doi.org/10.1371/journal.pone.0112391>.
- Young, O., T. Braggins, and G. Barker. 1994. Pyridinoline in ovine intramuscular collagen. *Meat Sci.* 37:297–303. [https://doi.org/10.1016/0309-1740\(94\)90088-4](https://doi.org/10.1016/0309-1740(94)90088-4).
- Zhang, W., B. M. Naveena, C. Jo, R. Sakata, G. Zhou, R. Banerjee, and T. Nishiumi. 2017. Technological demands of meat processing—An Asian perspective. *Meat Sci.* 132:35–44. <https://doi.org/10.1016/j.meatsci.2017.05.008>.