

Project Summary

New Beef Tenderness Theory

**Principal Investigators: Rhonda K. Miller, Stephen B. Smith, & Gordon
E. Carstens,
Texas A&M University**

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Background

Factors affecting beef tenderness continue to be a major concern for the beef industry. Meat scientists have examined the biochemical factors impacting beef tenderness for decades. One biochemical factor that affects beef tenderness is initial tenderness of beef immediately post-harvest and the subsequent improvement of tenderness with refrigerated storage, termed beef aging. It is well documented that beef is toughest approximately 24 hours post-harvest. With subsequent storage, beef becomes more tender. Beef from *Bos indicus*-influenced cattle have been shown to take longer to improve in tenderness post-harvest and in some cases, meat from these animals will not improve substantially in tenderness. Work by many researchers showed that calpains, enzymes located within the muscle cell, are capable of clipping or degrading structural proteins within the muscle. The action of calpains have been shown to be the same events that occur in post-harvest muscle or during meat aging and therefore, it is universally thought that calpains play a major role in improving beef tenderness during aging.

Another component of the calpain system is calpastatin. Calpastatin is the regulatory protein that controls the actions of calpains. The reason that meat aging takes multiple days, usually from 7 to 14 days, is that calpastatin initially keeps calpains, mainly μ -calpain, from acting freely. However, as calpastatin inhibits the action of calpains, they autolyse, or destroy themselves. Therefore, with time calpastatin levels decrease and allow calpains to work. While this seems like a very straight-forward explanation of the events that occur during meat aging, there still is a great deal of doubt among meat scientists that the system is this simple. It is well documented that calpains and calpastatin are stimulated or need free calcium in the sarcoplasm to work. Immediately post-harvest, calcium concentrations in the sarcoplasm increase. Calcium is stored in the sarcoplasmic reticulum of the muscle cell and after harvest, the sarcoplasmic reticulum cannot continue to hold calcium and calcium leaks out into the sarcoplasm. This increase in calcium stimulates the actions of calpains and calpastatin. Free calcium in the sarcoplasm also affects other biochemical changes. Once muscle is converted to meat, the muscle pH drops and temperature drops to levels where calpain and calpastatin are only minimally active at about 25%. As calpains and calpastatin autolyse or destroy themselves when they are active, there are very low levels of calpain and calpastatin within 72 to 96 hours post-harvest. As most of the improved tenderness occurs much later, it is logical that something in addition to calpain and calpastatin are contributing to improved tenderness with aging or something is working in concert with calpain and calpastatin.

More recently, evidence from this laboratory supports a new tenderness theory. Takahashi (1996) and Takahashi (1999) proposed that increased levels of free calcium in the sarcoplasm early post-harvest and during aging, while stimulating the actions of calpains and calpastatin, may simultaneously and just as importantly, be stimulating other biochemical events that improved meat tenderness with aging. It could be that meat tenderness is not only dependent on the level of calpastatin, but also the level of free calcium.

Researchers in Japan have shown that free sarcoplasmic calcium causes the breakdown or disassociation of paratropomyosin, desmin, titin and nebulin. These four proteins are important structural proteins that hold the muscle fiber matrix up. It has been universally accepted that desmin, titin and nebulin degrade post-harvest and that their degradation is an important component

of improvement in beef tenderness with aging. It is reasonable to hypothesize that calpain/calpastatin and the secondary theory of calcium mediated tenderization both could be contributing to meat tenderness during aging.

Objectives

- 1) Evaluate the role of level of sarcoplasmic free calcium on beef aging and improvements in tenderness.
- 2) Examine the interaction of sarcoplasmic free calcium and calpain activity in the degradative processes responsible beef tenderness with aging.

Methodology

Samples (n=169) were obtained from two unique groups of cattle fed at Texas A&M University and subsampled from a larger population of cattle that differed in tenderness. This was to create a data set with divergent tough and tender samples. One set of cattle was from a privately-funded source (n=89) where steers and heifers from years 2 and 3 of the project were used to select the most tender (n=45) and the toughest (n=44). Steers and heifers were fed a high-concentrate diet until an average fat thickness endpoint of 0.40 to 0.50 inches was reached. Calves were in south Texas and carcasses were electrically stimulated. Samples were obtained at 45 minutes, 24 hours, 10 days and 17 days post-harvest.

The second set of cattle were from a project funded by the Beef Checkoff were Santa Cruz yearling heifers (n=136) from the King Ranch. From this study, the 40 most tender and the 40 toughest carcasses were selected based on 14-day Warner-Bratzler shear force. Heifers were fed a dry-rolled corn-based finishing diet twice daily for a 150-day finishing period. Within tenderness and weight group, heifers were randomly assigned to one of four growth-enhancement treatments: (1) control (no implants and no beta-adrenergic agonist); (2) Revalor IH + Revalor H (**RV**; Intervet Inc.) with re-implant on day 70; (3) beta-adrenergic agonist (**B**; ractopamine, Optaflexx, TM Elanco Animal Health); and (4) RV + B. Heifers were fed to reach an approximate 0.4 inch fat thickness at the 12th rib. Heifers were harvested in south Texas and carcasses were electrically-stimulated as previously described. At 24 hours during chilling, an approximately 50 gm sample was removed for sarcomere length determinations.

After 48 hours, carcasses from both studies were split at the 12th rib and USDA Quality and Yield grade factors, lean color using a colorimeter and pH data were collected using trained evaluators. Carcasses were commercially fabricated and one strip loin was obtained from the left carcass side. Steaks, 1 inch thick, were cut from each strip loin and randomly assigned to aging time of 3, 10 or 17 days post-harvest. Steaks were individually vacuum-packaged and stored at 2°C until the specified aging period. Warner-Bratzler shear force was determined. A 0.5 inch steak was removed next to the 14-day Warner-Bratzler shear force steak for desmin determination. A 30 gm raw sample was removed from the dorsal portion of the raw *Longissimus* steak after 3, 10 and 17 d aging. This sample was used to determine desmin and titan degradation and calpain content. Collagen amount and solubility was determined from 17-day steaks only. A 25 to 30 gm sample was removed from the *Longissimus dorsi* and frozen in liquid nitrogen for sarcoplasmic-free calcium, sodium and potassium concentration from the 3-, 10- and 17-day aged steaks. Data were analyzed using the Proc Mixed and Proc Cor procedures of SAS (v9.2, SAS Institute, Inc., Cary, NC).

Findings

Tender steaks did not appreciably differ in carcass characteristics, sarcomere length, and collagen content from tough steaks. However, tender steaks had lower pH, and higher L^* , a^* and b^* color space values. These results indicate that pH and color measurements, while not explaining a high amount of variation in beef tenderness, are related to beef tenderness. These results continue to support the use of pH and color measurements in automated grading technology to predict beef tenderness.

The greatest differences between tough and tender steaks were in sarcoplasmic calcium concentration at 24 and 48 hours post-harvest, and desmin level after 17 days of aging. Tender steaks had higher calcium levels and lower desmin levels indicating that higher calcium levels stimulated protein degradation post-harvest. These results indicate that calcium flux from the sarcoplasmic reticulum either through the ryanodine receptor or thorough loss of membrane integrity drive differences in tenderness post-harvest.

Implications

Beef tenderness is becoming a more viable economic trait. The advent of automated grading to identify differences in tenderness and the success of brand-identified programs that pay a premium for tender beef are driving these economic changes. Selection of beef for tenderness has become a component for breeding programs and three companies have commercial genetic-markers that can assist beef producers in marker-assisted selection for beef tenderness. One of these companies has recently commercialized a second-generation genetic marker that utilizes much greater information on the genome to predict beef tenderness. As meat scientists, a great deal is known about beef tenderness, but when explaining variation in beef tenderness, a large volume of information is still not explained. Understanding what biological factors are being selected for using the commercial beef tenderness markers is somewhat known. For example, it is known that the commercial genetic markers for beef tenderness are highly dependent on the biology of the calpain/calpastatin system. This system has been shown to affect how rapidly and how much improvement in beef tenderness occurs with meat aging. Also, it is well known that meat aging affects beef tenderness. However, new evidence strongly supports that differences in how much calcium fluxes from the sarcoplasmic reticulum, the organelle inside the muscle fiber that holds calcium and helps in regulating how calcium is used for muscle contraction, also affects beef tenderness. Recent results from the Beef Checkoff-funded McGregor Gene-Mapping Project showed that tender families of cattle had higher sarcoplasmic calcium levels post-harvest than tough families.

Researchers took 168 steers and heifers that differed greatly in tenderness. These steers and heifers were selected from almost 2,000 steers and heifers from two projects so that we were assured that they represented the US beef supply and that they differed in tenderness. Factors that have been associated with differences in beef tenderness were measured and researchers found that tender steaks had higher sarcoplasmic calcium and that this effect most likely drove the calpain system to improve tenderness, especially at three days post-harvest. This higher sarcoplasmic calcium level in tender steaks also may have assisted in additional improvements in tenderness by assisting in degrading major structural proteins with subsequent post-harvest aging. It is most likely the combined effect that improved tenderness for “tender” steaks. These results help meat scientists to more fully understand factors driving differences in beef tenderness. Additionally, these results will assist those developing genetic selection tools, such as genetic-markers, to more fully understand the genetic regulation of the calcium channels in the sarcoplasmic reticulum and integrity of its membrane and the subsequent role in calcium flux post-harvest and beef tenderness.

Table 1. Least squares means for Warner-Bratzler shear force (kg) after 3, 10 and 17 days of post-harvest aging.

Effect	<u>Warner-Bratzler shear force, kg</u>		
	3 day	10 day	17 day
<u>Tenderness group^c</u>	<0.0001	<0.0001	<0.0001
Tough	4.53 ^b	3.66 ^b	3.70 ^b
Tender	2.94 ^a	2.45 ^a	1.83 ^a
RMSE ^c	1.006	0.827	0.516

^{ab}Mean values within a column and effect followed by the same letter are not significantly different (P > 0.05).

^cP-value from analysis of variance tables.

Table 2. Least squares means for sarcoplasmic calcium concentration (μ moles/liter) across aging times by tenderness group.

Effect	<u>Post-harvest aging time</u>					
	45 min	24 h	48 h	3 d	10 d	17 d
<u>Tenderness group^c</u>	0.69	0.01	0.02	0.14	0.80	0.29
Tough	146.99	109.21 ^a	98.07 ^a	144.36	217.78	183.26
Tender	126.35	380.52 ^b	177.05 ^b	182.84	236.91	255.02
Root mean square error	239.495	444.468	143.045	105.371	444.55	405.28

^{ab}Mean values within a column and effect followed by the same letter are not significantly different (P > 0.05).

^cP-value from analysis of variance tables.

For more information contact:

National Cattlemen's Beef Association
 9110 East Nichols Avenue
 Centennial, Colorado 80112-3450
 (303) 694-0305