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## Native Beef Collagenase MMP-9 May Contribute to Tenderness Improvement by Degrading Connective Tissues in Extended Aged Beef

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# Native Beef Collagenase MMP-9 May Contribute to Tenderness Improvement by Degrading Connective Tissues in Extended Aged Beef

## Abstract

**Objective:** Collagen is one of the main components in the connective tissue (CT) and contributes to background toughness in beef. It is known that in living animals, collagen can be degraded and remodeled by collagenase matrix metalloproteinases (MMP); however, it is unclear if collagenase MMP can impact CT texture during postmortem aging of beef. Therefore, this study aimed to understand how collagenase MMP activity may impact postmortem connective tissue degradation in beef in three different cuts and four different aging periods.

**Study Description:** Beef boneless striploin, top sirloin butt, and heel were acquired from 10 U.S. Department of Agriculture high choice beef carcasses and assigned to be aged for 3, 21, 42, or 63 days ( $n = 120$ ). Following each aging time, Warner-Bratzler shear force (WBSF), connective tissue shear force (CTSF), trained panel responses, collagen content, denaturation temperature of connective tissue, collagen crosslinks density, connective tissue degradation product, and native collagenase activity were measured, and collagenase identity was identified as MMP-9 through Western blot.

**Results:** Striploin was considered the most tender muscle ( $P < 0.01$ ), and tenderness was improved ( $P < 0.01$ ) after 21 days of aging. In addition, CTSF data and trained panelists demonstrated softening ( $P < 0.05$ ) of CT after 21 days of aging. Heel and top sirloin butt did not differ ( $P > 0.10$ ) in collagen content and had greater ( $P < 0.01$ ) collagen content than striploin. However, no aging effect was found for collagen content ( $P > 0.10$ ). Denaturation temperature of CT decreased and collagen crosslinks density increased after 42 days of aging for all cuts evaluated in this study ( $P < 0.01$ ). The MMP-9 activity decreased ( $P < 0.01$ ) from 3 to 21 to 42 days, and it had the greatest ( $P < 0.01$ ) activity in heel compared to the other two cuts. Heel and striploin had greater ( $P < 0.01$ ) connective tissue degradation product than top sirloin butt. It was interesting to note that while striploin and heel showed a decrease ( $P < 0.05$ ) in the degradation product from 3 to 21 to 42 days, top sirloin butt did not show any changes ( $P > 0.10$ ) in degradation product during the entire 63 days of aging period.

**The Bottom Line:** These results provide an explanation on CT softening during postmortem aging. Understanding the mechanism of tenderness improvement from the softening of CT may help the industry improve the eating quality of lower quality beef cuts.

## Keywords

connective tissue, matrix metalloproteinases, tenderness

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## Cover Page Footnote

The Beef Checkoff.

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### Abstract

The objective of this study was to characterize native beef collagenase activity and understand how its activity may impact postmortem connective tissue degradation. Beef boneless striploin, top sirloin butt and heel were acquired from ten U.S. Department of Agriculture high choice beef carcasses, fabricated into steaks and aged for 3, 21, 42, and 63 days. Steaks from each aging period from each subprimal were assigned to instrumental and sensory tenderness analyses, collagen biochemical analysis, collagenase activity characterization, and collagenase identification. Heel and top sirloin butt were considered tougher than striploin. After 21 days of aging, overall steaks and connective tissue texture become more tender. Collagen content did not change ( $P > 0.10$ ) in the different aging periods. Denaturation temperature of connective tissue decreased ( $P < 0.01$ ) after 42 days of aging. In contrast, collagen crosslink density increased ( $P < 0.05$ ) after 42 days of postmortem aging. Striploin had a decrease ( $P < 0.05$ ) in the connective tissue (CT) degradation product from 3 to 21 to 42 days, and heel had a reduction ( $P < 0.01$ ) in the CT degradation product from 3 to 42 days. A collagenase at 72 kDa was identified as matrix metalloproteinase-9 (MMP-9) through Western blot. The MMP-9 activity was detected throughout the different muscles and aging periods and had the greatest activity at three days of aging, which decreased ( $P < 0.01$ ) from 3 to 21 to 42 days.

### Introduction

Beef tenderness is mostly dictated by myofibrillar protein and connective tissue properties with the former receiving much greater research focus than the latter. Collagen is one of the main components in connective tissue (CT) and contributes to background toughness in beef. It is known that in living animals, collagen can be degraded and remodeled by collagenase matrix metalloproteinases (MMP); however, it is unclear if collagenase MMPs can impact CT texture during postmortem aging of beef. Therefore, this study aimed to characterize native beef collagenase activity and understand how its activity may impact tenderness by postmortem connective tissue degradation in three different beef cuts and four different aging periods.

## Experimental Procedures

Beef boneless striploin, top sirloin butt, and heel from both sides were acquired from ten U.S. Department of Agriculture high choice beef carcasses. Each muscle was fabricated into steaks and aged at 35.6°F for four different aging periods: 3, 21, 42, and 63 days. Warner-Bratzler shear force (WBSF), connective tissue shear force (CTSF), and evaluations by trained panelists were performed to measure tenderness. Native collagenase activity was measured by zymography gels casted with bovine type I collagen, and collagenase identity was identified as MMP-9 through Western blot. To identify changes in intramuscular connective tissue (IMCT) components, collagen content, denaturation temperature of CT, collagen crosslinks density, and CT degradation product were performed.

## Results and Discussion

It was expected to find heel and top sirloin butt being tougher than striploin ( $P < 0.01$ ; Table 1). Cuts from muscles used for locomotion usually have a greater proportion of connective tissue due to their needs to sustain greater force in their functions. This research demonstrated a decrease in connective tissue amount detected by the trained panelists ( $P < 0.05$ ; Table 2) and CTSF ( $P < 0.05$ ; Table 3) during the aging process. These results are evidence indicating that CT can also go through a weakening/degradation process during postmortem aging. Locomotive muscles had more ( $P < 0.01$ ) collagen content than supportive muscles as expected; however, no changes in collagen content during the aging period was detected ( $P > 0.10$ ). However, an increase in mature collagen crosslink density was detected ( $P < 0.05$ ) with extended aging period, but denaturation temperature of connective tissue decreased ( $P < 0.01$ ) with advanced aging time. This was unexpected as mature collagen crosslinks are known to be thermally stable and likely contribute to meat toughness. This finding indicates that there may be modifications in other connective tissue components or other type of collagen crosslinks. The MMP-9 was identified at the 72 kDa band in the zymography gels (Figure 1). Evidence is provided in this study that collagen is being modified by MMPs as MMP-9 activity was found in beef from all four aging periods evaluated. Moreover, there was a decrease ( $P < 0.01$ ) in MMP-9 activity after extended aging, which might be explained by the gradual inactivation of endogenous enzymes throughout the aging process. Finally, CT degradation product decreased ( $P < 0.01$ ) during aging, and this phenomenon could also suggest a further breakdown occurring. Although CT degradation product is independent of collagen degradation, this noted degradation is also due to the action of MMPs.

## Implications

This study showed that IMCT is going through some changes that softened IMCT during postmortem aging, and MMP-9 is active in postmortem beef muscles. Understanding this mechanism may assist the beef industry to improve tenderness in lower quality beef cuts through the manipulation of MMP-9 activity in beef.

## Acknowledgment

The Beef Checkoff.

**Table 1. Main effect of beef cut for Warner-Bratzler shear force (WBSF), connective tissue amount and overall tenderness evaluated by the trained panelists, collagen content, and MMP-9 activity of three different beef muscles aged for 3, 21, 42, or 63 days (n = 120)**

Items	Muscle			SEM <sup>1</sup>	P-value
	Boneless striploin	Top sirloin butt	Heel		
WBSF, lb	7.52 <sup>b</sup>	9.68 <sup>a</sup>	9.46 <sup>a</sup>	0.14	< 0.01
Connective tissue amount scores <sup>2</sup>	12.72 <sup>b</sup>	16.68 <sup>a</sup>	17.93 <sup>a</sup>	1.24	< 0.01
Overall tenderness scores <sup>3</sup>	60.28 <sup>a</sup>	50.28 <sup>b</sup>	57.94 <sup>a</sup>	1.26	< 0.01
Collagen, %	0.50 <sup>b</sup>	0.80 <sup>a</sup>	0.91 <sup>a</sup>	0.82	< 0.01
MMP-9, fold change	0.80 <sup>b</sup>	1.04 <sup>b</sup>	1.91 <sup>a</sup>	0.14	< 0.01

<sup>a,b</sup> Values within a row with different superscripts differ significantly at  $P < 0.05$ .

<sup>1</sup>SEM = Standard error of the mean.

<sup>2</sup>Connective tissue scores: 0 = none; 100 = extremely abundant.

<sup>3</sup>Overall tenderness scores: 0 = extremely tender; 50 = neither tender or tough; 100 = extremely tough.

**Table 2. Main effect of aging period for Warner-Bratzler shear force (WBSF), connective tissue amount, and overall tenderness evaluated by the trained panelists, transition temperature of connective tissue, collagen crosslink density, and MMP-9 activity of three different beef muscles aged for 3, 21, 42, or 63 days (n = 120)**

Items	Aging (days)				SEM <sup>1</sup>	P-value
	3	21	42	63		
WBSF, lb	10.32 <sup>a</sup>	8.66 <sup>b</sup>	8.58 <sup>b</sup>	8.02 <sup>b</sup>	0.13	< 0.01
Connective tissue amount score <sup>2</sup>	19.44 <sup>a</sup>	14.01 <sup>b</sup>	13.87 <sup>b</sup>	14.34 <sup>b</sup>	1.69	< 0.05
Overall tenderness score <sup>3</sup>	50.69 <sup>b</sup>	56.12 <sup>a</sup>	58.45 <sup>a</sup>	59.43 <sup>a</sup>	1.52	< 0.01
Denaturation temperature of collagen, °F	149.74 <sup>a</sup>	149.92 <sup>a</sup>	148.71 <sup>b</sup>	148.5 <sup>b</sup>	0.20	< 0.01
Collagen crosslink density, mol/mol	0.13 <sup>b</sup>	0.14 <sup>ab</sup>	0.16 <sup>a</sup>	0.16 <sup>a</sup>	0.01	< 0.05
MMP-9, fold change	2.46 <sup>a</sup>	1.05 <sup>b</sup>	0.72 <sup>c</sup>	0.75 <sup>c</sup>	0.12	< 0.01

<sup>a,c</sup> Values within a row with different superscripts differ significantly at  $P < 0.05$ .

<sup>1</sup>SEM = Standard error of the mean.

<sup>2</sup>Connective tissue amount scores: 0 = none; 100 = extremely abundant.

<sup>3</sup>Overall tenderness scores: 0 = extremely tender; 50 = neither tender or tough; 100 = extremely tough.

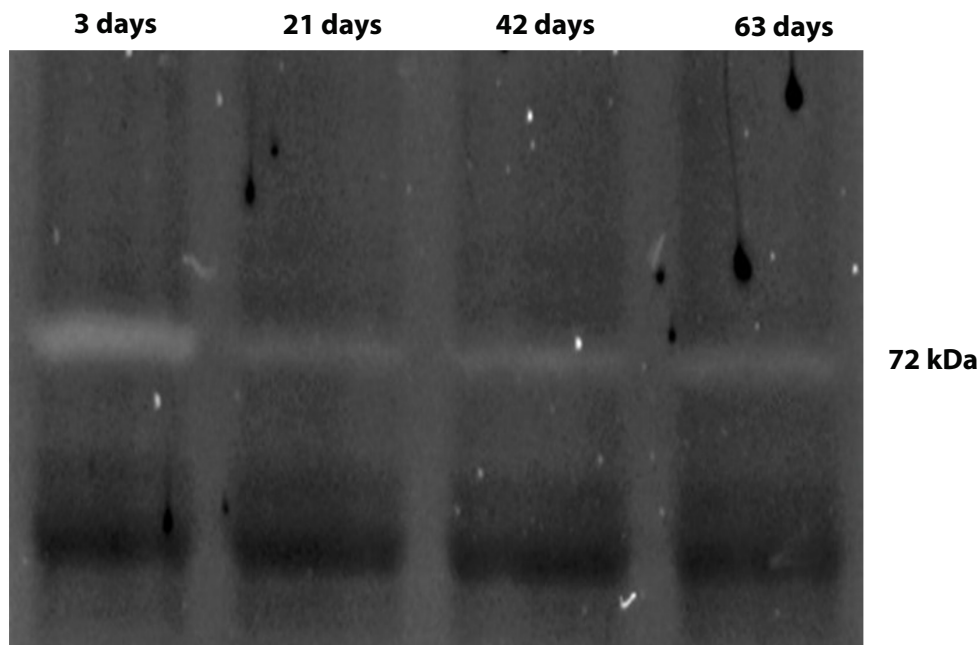
**Table 3. Connective tissue shear force (CTSF) and aggrecan fragmentation of three different beef muscles aged for 3, 21, 42, or 63 days (n = 120)**

Items	Age (days)	Muscles			SEM <sup>1</sup>	<i>P</i> -value
		Boneless striploin	Top sirloin butt	Heel		Muscle × age
CTSF, lb	3	7.41 <sup>Aa</sup>	7.67 <sup>Aa</sup>	8.44 <sup>Aa</sup>	0.18	< 0.05
	21	6.55 <sup>ABa</sup>	7.19 <sup>Aa</sup>	6.75 <sup>Ba</sup>		
	42	5.95 <sup>Bb</sup>	8.16 <sup>Aa</sup>	7.19 <sup>Ba</sup>		
	63	5.51 <sup>Bb</sup>	7.39 <sup>Aa</sup>	7.25 <sup>Ba</sup>		
CT degradation product, fold change	3	0.77 <sup>Aa</sup>	0.09 <sup>Ab</sup>	0.70 <sup>Aa</sup>	0.1	< 0.01
	21	0.50 <sup>Ba</sup>	0.02 <sup>Ab</sup>	0.52 <sup>ABa</sup>		
	42	0.17 <sup>Ca</sup>	0.04 <sup>Aa</sup>	0.29 <sup>Ba</sup>		
	63	0.06 <sup>Ca</sup>	0.04 <sup>Aa</sup>	0.22 <sup>Ba</sup>		

<sup>a-b</sup>Within a row, means without a common superscript differ at  $P < 0.05$ .

<sup>A-B</sup>Within a column of one item, means without a common superscript differ at  $P < 0.05$ .

<sup>1</sup>SEM = Standard error of the mean.



**Figure 1. Representative image of MMP collagenase identified by collagen zymography in beef boneless striploin.**