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# Investigating the Contribution of Mature Collagen Crosslinks to Cooked Meat Toughness Using a Stewed Beef Shank Model

W. Wu, A.A. Welter, C.K.Y. Chun, T.G. O'Quinn, G. Magnin-Bissel, and M.D. Chao

### Abstract

The objective of this study was to investigate mature collagen crosslink densities and their relationship to connective tissue texture using a stewed beef shank model. Six shank cuts, three from forequarter [biceps brachii (shank A); deep digital flexor from foreshank (shank B); extensor carpi radialis (shank C)], and three from hindquarter [flexor digitorum superficialis (shank D); deep digital flexor from hindshank (shank E); a combination of long digital extensor, medial digital extensor, and peroneus tertius (shank F)] were collected from eight U.S. Department of Agriculture Low Choice beef carcasses. Shanks from the left side of the carcasses were designated for the cooked treatment, and shanks from the right side were designated as the raw treatment. Connective tissue texture, Warner-Bratzler shear force, and collagen content and characteristics were measured for the six different beef shank cuts. Shank B had the toughest connective tissue texture, greatest Warner-Bratzler shear force value, most cooked collagen content, greatest insoluble collagen percentage as well as greatest raw and cooked pyridinoline densities among all the beef shank cuts (P < 0.05). Correlation analysis showed that cooked collagen content, insoluble collagen percentage as well as raw pyridinoline densities had positive correlations with connective tissue texture (r = 0.550, 0.498, and 0.560 respectively; P < 0.01) and Warner-Bratzler shear force (r = 0.615, 0.392, and 0.730, respectively; P < 0.05). Raw pyridinoline density may be a good indicator for cooked beef connective tissue texture and ultimately, tenderness in beef cuts with a high concentration of connective tissue prepared with moist heat cookery.

### Introduction

It is well established that connective tissue provides the "background toughness" in meat, and past research has demonstrated this background toughness is the result of heat insoluble collagen content in meat after cooking. However, the characteristics of heat insoluble collagen are not well studied. Therefore, the objective of this study was to investigate mature collagen crosslink densities and their relationship to cooked beef tenderness and connective tissue texture using a stewed beef shank model.

### **Experimental Procedures**

The cross-section and whole-muscle cut of six different beef shank cuts, three from the forequarter [biceps brachii (shank A); deep digital flexor from foreshank (shank B); extensor carpi radialis (shank C)] and three from the hindquarter [flexor digitorum superficialis (shank D), deep digital flexor from hindshank (shank E), a combination of long digital extensor, medial digital extensor, and peroneus tertius (shank F)] that were collected from eight USDA Low Choice beef carcasses (n = 48) are shown in Figure 1. Shanks from the left side of the carcasses were designated for the cooked treatment, then were stewed in water for 90 minutes at 199°F, and shanks from the right side were designated as the raw treatment. Asian consumers (n = 61) evaluated the connective tissue texture from cooked shanks based on Just About Right line scales. Since connective tissue texture is an important component of many Asian cuisines, only Asian consumers were selected for the consumer panel of this study due to their ability to distinguish small differences in connective tissue texture. In addition, Warner-Bratzler shear force value was obtained. Mature collagen crosslinks densities (pyridinoline and deoxypyridinoline) and collagen content were measured for raw and cooked beef shanks. The collagen contents were adjusted to dry matter basis based on moisture content of the raw and cooked shanks to account for moisture loss during the cooking process, and the relative percentages of soluble and insoluble collagen content were calculated. Finally, a correlation analysis was performed to understand the relationship between each collagen characteristic and cooked beef shank tenderness.

### **Results and Discussion**

Collagen content results are displayed in Table 1. There was a significant muscle × cooking treatment interaction for collagen content (P < 0.01). In general, shanks D and F had higher amount of raw collagen content compared to A and E (P < 0.01). Shank B had higher amount of raw collagen than shank C (P < 0.01), but it was not different from the other four beef shank cuts (P > 0.05). Shank C contained the least amount of raw collagen among all the beef shank cuts (P < 0.01). On the other hand, all the beef shank cuts had similar cooked collagen content except for shank B (P < 0.01), which had the greatest cooked collagen content. In addition, collagen content declined after cooking for all the beef shank cuts (P < 0.01).

Results for soluble and insoluble collagen percentages, connective tissue texture evaluated by Asian consumers, and Warner-Bratzler shear force are shown in Table 2. Shanks A, D, E, and F all had greater soluble and least insoluble collagen percentage compared to shank B (P < 0.01), while shank C was in between and not different from shanks A, B, E, and F (P > 0.05) for insoluble collagen. Among all the beef shank cuts evaluated in this study, Asian consumers rated shank B with the toughest connective tissue texture, followed by shank E, with shanks A and D having the softest connective tissue texture among all (P < 0.01). Shanks C and F had softer connective tissue texture than shank B, but were not different from shanks A, D, and E (P > 0.05). Finally, shank B was significantly tougher than the rest of shank cuts when measured by Warner-Bratzler shear force (P < 0.01), and all other beef shank cuts had much lower but similar Warner-Bratzler shear force values (P > 0.10).

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Raw and cooked pyridinoline and deoxypyridinoline density of the six different beef shank cuts are shown in Table 3. There was a significant muscle × cooking treatment interaction for pyridinoline density. Cooking only decreased pyridinoline density for shank B (P < 0.05), and pyridinoline density for the rest of the beef shank cuts was not affected by cooking (P > 0.10). For deoxypyridinoline density, Shank C was one of the beef shank cuts that had greater deoxypyridinoline density for raw and cooked samples (P < 0.01). Again, there was a cooking effect in which cooking decreased deoxypyridinoline density for shanks B, C, and D (P < 0.01), but not for the other cuts (P > 0.10).

Correlation coefficients of raw and cooked collagen content, soluble and insoluble collagen percentage, and different collagen crosslinks density with connective tissue texture and Warner-Bratzler shear force of six different beef shanks are presented in Table 4. As expected, cooked collagen content, insoluble collagen percentage as well as raw pyridinoline densities had positive correlations with connective tissue texture (P < 0.01) and Warner-Bratzler shear force (P < 0.05). There was still a noted positive correlation between cooked pyridinoline density with connective tissue texture (P < 0.05) and Warner-Bratzler shear force (P < 0.10), but the relationship was not nearly as strong as for raw pyridinoline density.

### Implications

Pyridinoline is a heat stable collagen crosslink, and raw pyridinoline density is a good indicator for heat insoluble collagen content, cooked beef connective tissue texture and ultimately, tenderness in beef cuts with a high concentration of connective tissue prepared with moist heat cookery.

	Collagen content, % dry matter basis <sup>1</sup>		Standard error of the least		
<b>Beef shank</b>	Raw	Cooked	squares means	P-value	
Foreshank			4.21	< 0.01	
А	5.74 <sup>Ab</sup>	3.08 <sup>Bb</sup>			
В	6.54 <sup>Aab</sup>	$4.71^{Ba}$			
С	4.22 <sup>Ac</sup>	2.70 <sup>Bb</sup>			
Hindshank					
D	$7.58^{Aa}$	3.23 <sup>Bb</sup>			
E	5.71 <sup>Ab</sup>	3.12 <sup>Bb</sup>			
F	7.58 <sup>Aa</sup>	3.55 <sup>Bb</sup>			

Table 1. Least square means of raw and cooked collagen content of the six different beef shank cuts (n = 48)

<sup>abc</sup>Least squares means in a column without a common superscript differ (P < 0.05).

<sup>AB</sup>Least squares means in a row without a common superscript differ (P < 0.05).

<sup>1</sup>Raw and cooked collagen content were adjusted to dry matter basis to account for moisture loss during the cooking process.

<b>Beef shank</b>	Soluble collagen, % <sup>1</sup>	Insoluble collagen, % <sup>1</sup>	Connective tissue texture <sup>2</sup>	Warner-Bratzler shear force, lb
Foreshank	-	_		
А	45.58 <sup>ab</sup>	54.42 <sup>bc</sup>	47.90°	7.27 <sup>b</sup>
В	27.70°	$72.30^{a}$	75.54ª	19.51ª
С	35.95 <sup>bc</sup>	64.05 <sup>ab</sup>	52.13 <sup>bc</sup>	7.30 <sup>b</sup>
Hindshank				
D	57.16ª	42.84 <sup>c</sup>	45.23°	8.60 <sup>b</sup>
E	43.31 <sup>ab</sup>	56.69 <sup>bc</sup>	55.89 <sup>b</sup>	8.05 <sup>b</sup>
F	51.94 <sup>ab</sup>	48.06 <sup>bc</sup>	51.55 <sup>bc</sup>	8.58 <sup>b</sup>
Standard error of the	5.38	5.38	2.60	0.62
least squares means				
<i>P</i> -value	0.01	0.01	< 0.01	< 0.01

Table 2. Least squares means of percent soluble and insoluble collagen, connective tissue
texture evaluated by Asian consumers, <sup>3</sup> and Warner-Bratzler shear force of six different
beef shanks $(n = 48)$

<sup>abc</sup>Least squares means in a column without a common superscript differ (P < 0.05).

 $^{1}$ Soluble collagen % = (raw collagen content – cooked collagen content) / raw collagen content.

Insoluble collagen % = cooked collagen content / raw collagen content – all in dry matter basis.

<sup>2</sup>Connective tissue texture scores: 0 = too soft; 50 = just about right (ideal score); 100 = too hard.

<sup>3</sup>Asian consumers were selected for the consumer panel of this study due to their ability to distinguish small differences in connective tissue texture.

Table 3. Least square means of raw and cooked pyridinoline and deoxypyridinoline densities of six different beef shanks (n = 48)

	Pyridinoli mol/mo	ne density, l collagen	Standard error of the least		Deoxypy density, mol	ridinoline /mol collagen	Standard error of the least	
Beef shank	Raw	Cooked	square means	P-value	Raw	Cooked	square means	<i>P</i> -value
Foreshank			0.04	< 0.05			0.001	< 0.01
А	$0.14^{Ac}$	0.23 <sup>Ab</sup>			0.008 <sup>Ac</sup>	$0.012^{Aa}$		
В	$0.54^{Aa}$	$0.42^{Ba}$			0.016 <sup>Aa</sup>	0.008 <sup>Bb</sup>		
С	0.19 <sup>Ac</sup>	$0.14^{Ac}$			0.019 <sup>Aa</sup>	$0.013^{Ba}$		
Hindshank								
D	$0.34^{Ab}$	0.28 <sup>Ab</sup>			$0.014^{Ab}$	$0.007^{Bb}$		
E	0.19 <sup>Ac</sup>	0.31 <sup>Ab</sup>			0.010 <sup>Ac</sup>	$0.010^{Aa}$		
F	0.13 <sup>Ac</sup>	0.12 <sup>Ac</sup>			0.010 <sup>Ac</sup>	0.007 <sup>Ab</sup>		

<sup>abc</sup>Least squares means in a column without a common superscript differ (P < 0.05).

<sup>AB</sup>Least squares means in a row without a common superscript differ (P < 0.05).

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	Connective tissue	Warner-Bratzler
Collagen components	texture	shear force
Raw collagen content in dry matter basis <sup>1</sup>	-0.005	0.211
Cooked collagen content in dry matter basis <sup>1</sup>	0.550***	0.615***
Soluble collagen %	-0.498***	-0.392**
Insoluble collagen %	0.498***	0.392**
Raw pyridinoline density	0.560***	0.730***
Cooked pyridinoline density	0.375**	0.324*
Raw deoxypyridinoline density	0.257	0.321*
Cooked deoxypyridinoline density	-0.150	-0.220

Table 4. Correlation coefficient (r) of raw and cooked collagen content, soluble and insoluble collagen % and raw and cooked mature collagen crosslink densities with connective tissue texture and Warner-Bratzler shear force of six beef shanks

<sup>1</sup>Raw and cooked collagen content were adjusted to dry matter basis to account for moisture loss during the cooking process.

\* P < 0.10.

\*\* P < 0.05.

\*\*\* P < 0.01.