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6 : 23

EFFECT OF CONNECTIVE TISSUES ON THE SENSORY TEXTURAL SCORES AND SHEAR FORCES OF RESTRUCTURED BEEF STEAKS

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SUMMARY

Restructured beef steaks made from cheaper forequarter muscles tend to be unacceptable upon chewing because of detectable connective tissues. This study was designed to determine and characterize an acceptable level of various connective tissues and then develop chemical and enzymatic degradation methods to reduce objectionable connective tissue burdens to an acceptable range.

Highly acceptable restructured beef steaks can be made from forequarter muscles if the tendon is removed and the epimysium and gristle are minimized. The latter two should be present at less than approximately 3 mg hydroxyproline per g steak.

Treatment of the surface of intact muscles to degrade the epimysium with lactic acid was demonstrated to reduce the objectionable connective tissue. Collagenase treatments were also shown to have potential.

OBJECTIVE

Restructured beef steaks have been made with acceptable binding, color and flavor, but when made from more economical portions of the carcass, such as the chuck, they have an unacceptable texture (Booren et al., 1981, Berry et al., 1986). If the flake size is small, the connective tissue is not detectable; texture is a function of the particle size and binding. However, this product lacks a steak-like texture. Restructured steaks made with larger pieces of meat have a more desirable texture during the initial bites, but upon continuous chewing, an unacceptable bolus of connective tissue remains in the mouth. Extensive hand trimming effectively removed connective tissue but was uneconomical (Recio et al., 1986). The objectives of this research are to 1) correlate the relative contributions of tendon, epimysium, gristle and peri-/endomysial tissues to the objectionable characteristics of restructured beef and 2) find ways to reduce the perception of the connective tissue by chemical or enzymatic treatments.

METHODS

Meat USDA Choice,yield grade 2, subprimal cuts of chuck, clod and rib were obtained from a local distributor. The muscles were excised and stored at 1°C.

Product

Series A: Four chucks were quantitatively trimmed of tendon, epimysium, gristle and adipose tissues. A perimysium/endomysium reticulum extract was made by extracting myofibrillar and sarcoplasmic proteins out of thinly sliced muscle with a series of $CaCl_2$/NaCl solutions. The trimmed muscles were diced (1 cm) and varying amounts of the connective tissues (0.5 cm dice) were added. They were mixed for 2 min with 0.75% NaCl and 0.12% sodium tripolyphosphate, stuffed into 5.5 cm casings (Series A and D) or packed under vacuum into loaf pans (Series B & C), frozen, sliced into 2.5 cm steaks, vacuum packaged and stored frozen.

Series B: Supraspinatus and Infraspinatus muscles from two clods were excised, frozen, sliced 3 mm thick across the fibers and recut into three portions that contained epimysium, gristle or neither. Restructured steaks were made as described above.

Series C: Rhomboideus and Triceps brachii-Serratus ventralis muscles from the chuck and Longissimus dorsi from the rib of two animals were excised, trimmed free of epimysium and gristle, frozen and sliced (3 mm) parallel or perpendicular to the fiber direction. The muscles were considered to be tough, moderate and tender, respectively, and six treatments were made as described above.

Series D: The Infraspinatus, Supraspinatus and Triceps brachii were excised from three clods. Each muscle was divided into thirds and randomly assigned to be 1) untrimmed control, 2) extensively trimmed control or 3) treated with 0.5 M lactic acid at 20°C for 30 min and then rinsed with water. The meat was diced and made into restructured steaks as described above.

Series E: Flaked chuck, microbial collagenases (20 mg per 200 g meat) and α-amylase (10 mg per 200 g), 0.5% NaCl and 0.25% sodium tripolyphosphate were mixed together, stuffed (3.8 cm casings), frozen and sliced (1.2 cm) and vacuum sealed. Thawed slices were cooked in a water bath at 65°C for 30 min to simulate cooking. Microbial collagenases were from Clostridium histolyticum, Achromobacter iophagus and Vibrio B-30.

Analyses

Sensory panel Steaks were partially thawed and cooked on an electric grill (turned once) until the center reached 70°C. In series A, B and C, a trained panel scored initial tenderness, connective tissue after chewing and overall texture on a 10.0 cm unstructured line. A score of 10 was given to highest initial tenderness, minimal connective tissue, and highest overall texture. In series D, an 85 member consumer panel scored product for flavor, connective tissue and overall texture with a score of 10 for most desirable flavor, minimal connective tissue, and highest overall texture.

Shear Force: An Instron Universal Testing Machine with the Lee-Kramer multiple-blade cell was used on uncooked and cooked restructured steaks (charred surface removed). Values were calculated as Newtons per g steak.

Hydroxyproline: Determinations to estimate collagen and connective tissue were made on carefully homogenized samples by the technique of Woessner (1961) and reported as mg hydroxyproline per g steak. The percentage of hydroxyproline solubilized by collagenases was determined (series E) by homogenizing (Polytron) in 1 mM EDTA and centrifuging. Supernatant and pellet were individually lyophilized and analyzed for hydroxyproline content.

Sarcomere length (μ) was determined by laser diffraction in gluteraldehyde fixed samples. The pH was measured in water slurries of raw steaks.

RESULTS

Series A (Table 1) showed the panel rejected relatively low levels of tendon but did not perceive peri/endomysial tissue. Higher levels of epimysium and gristle were given significantly lower scores for connective tissue and overall texture but not for initial tenderness. The shear force values on grilled steaks showed little relationship to added connective tissue (r = -.11, n = 40) but sensory connective tissue scores significantly correlated with shear forces on raw steaks (r = -.50, n = 40) and the hydroxyproline values (r = -.56, n = 40).

Series B (Table 2) had the epimysium and gristle in their natural relationship with the muscles. These steaks received lower sensory scores and had higher raw shear force values and hydroxyproline. Correlation coefficient between connective tissue score and hydroxyproline content was -.76 and between shear force and hydroxyproline was 0.93.

Series C (Table 3) indicated that long fibers (sliced parallel) or short (sliced perpendicularly) did not affect sensory perceptions of texture and only slightly affected shear forces. Both panel scores and shear forces indicated the expected decrease in connective tissue scores in steaks made with Rhomboideus. Initial tenderness scores were also lower with Rhomboideus. The hydroxyproline values were low compared to series A and B and Rhomboideus values were inconsistent. Sarcomere lengths did not reveal major differences. Sensory connective tissue scores were correlated to shear force (r = -.95) and hydroxyproline content (r = -.62).

Series D (Table 4) showed that the trimmed control steaks had significantly reduced shear forces and better sensory scores than the untrimmed controls. Muscles whose surface was treated with lactic acid had shear values and sensory texture scores between the two control treatments. Flavor was not affected by the lactic acid but pH values were reduced and cooking losses increased.

Series E (Table 5) demonstrated that addition of collagenases significantly increased the percentage of soluble collagen. α-Amylase aided the solubilization of collagen with A. iophagus.

DISCUSSION

Shear force measurements on the raw restructured steaks effectively measure connective tissue and correlate with sensory panel data. In the cooked steaks, collagen partially gelatinized and myofibrillar proteins coagulated, obscuring the connective tissue present (Moller, 1980). However, panelists still could perceive the connective tissue in cooked steaks.

Panelists strongly objected to the tendon but did not object to perimysium and endomysium. Gristle and epimysium were scored similarly and the addition of 1 mg hydroxyproline (11 mg collagen) per g steak reduced the sensory score approximately 1 unit on the 10 unit scale. Therefore, hydroxyproline line and shear force values can predict how a product will be perceived by the consumer. The similarity of responses for steaks made from chunks (Series A) and slices (Series B and C) indicated these conclusions apply to a range of sizes of meat.

Unexpectedly, muscle fiber direction had no effect on sensory or shear force values. Steaks made from Rhomboideus were significantly tougher although hydroxyproline values did not show consistently greater amounts of peri- and endomysium. The myofibrillar component of texture must be as evident in 3 mm sections of fiber as in 1 cm sections. All sensory scores were relatively good when epimysium was removed (Table 3) compared to scores in Tables 1 and 2 where they were present, demonstrating the importance of epimysium and gristle in determining textural acceptability in restructured steaks.

Lactic acid (and probably other acids) can disrupt and solubilize collagen and thereby reduce the perceived toughness of the epimysial connective tissues without causing complete loss of functional and sensory qualities of the underlying meat. The uncooked, acid treated, frozen steaks had brown areas but this was not noticeable after cooking. Binding of water and adhesion between meat chunks in the acid treated steaks was poorer than the controls, but using calcium alginate to bind the steaks would probably improve binding and adhesion (Means et al., 1987).

Microbial collagenases were capable of disrupting the connective tissues under realistic conditions. The amylases may help disrupt the glycoprotein matrix permitting more effective collagenase activity. Research is continuing to determine shear properties after enzyme treatment (sensory panels can not be used with these enzymes because their safety is unproven). Treatment of the epimysium by these enzymes and other proteases will also be evaluated.

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TABLE 1. Texture of Restructured Steaks with Added Connective Tissues

	Control	Tendon		Peri/endomysium		Epimysium		Gristle	
		2.5%	5.0%	5.0%	10.0%	5.0%	10.0%	5.0%	10.0%
Sensory Scores[a]									
Initial Tenderness	6.42	5.53*	4.98*	5.91	6.36	6.59	5.82	6.06	5.78
Connective Tissue	6.42	4.52*	3.48*	6.32	5.99	6.47	4.96*	5.76	4.94*
Overall Texture	6.22	4.41*	3.84*	6.01	5.75	6.36	5.17*	5.48	4.99*
Hydroxyproline (mg/g)	1.12	2.00*	3.00*	1.18	1.34	2.58*	3.48*	2.34*	4.04*
Shear Force (Newtons/g)									
Raw	62	71	84*	62	60	80*	91*	82*	98*
Cooked	79	84	87*	79	79	79	75	77	73

*Significantly different from control ($p \leq 0.05$)

[a] Lowest or least desirable score was 0.0, highest or most desirable was 10.0

Table 2. Texture of Restructured Steaks from Slices Containing Epimysium and Gristle

	Control	Gristle	Epimysium
Sensory Scores[a]			
Initial Tenderness	7.7^{ab}	7.9^{b}	6.8^{a}
Connective Tissue	7.6^{b}	5.9^{a}	4.8^{a}
Overall Texture	7.3^{b}	6.2^{ab}	5.4^{a}
Shear Force (Newtons/g)	40^{a}	56^{b}	77^{c}
Hydroxyproline (mg/g)	0.91^{a}	3.30^{b}	4.18^{c}

Means in a row with the same superscript are not significantly different ($p \geq 0.05$)

[a] Same as Table 1

Table 3. Muscle and Myofiber Direction

	Longissimus		Triceps-Serratus		Rhomboideus	
	//[a]	⊥[b]	//	⊥	//	⊥
Sensory Scores[c]						
Initial Tenderness	8.0^{a}	7.8^{a}	7.5^{ab}	7.4^{ab}	6.6^{bc}	6.0^{c}
Connective Tissue	8.6^{a}	8.4^{a}	8.0^{a}	8.0^{a}	6.7^{b}	6.3^{b}
Overall Texture	8.0^{a}	7.6^{a}	7.6^{a}	7.5^{a}	6.5^{b}	5.9^{b}
Shear Force (Newtons/g)	29^{ab}	25^{a}	34^{b}	40^{c}	59^{d}	64^{d}
Hydroxyproline (mg/g)	0.84^{ab}	0.80^{a}	0.92^{b}	0.78^{a}	0.89^{ab}	1.72^{c}
Sarcomere Length (μ)	1.82		2.34		1.89	

Means within a row with the same superscript are not significantly different ($p \geq 0.05$)

[a] Sliced parallel to myofibril [b] Sliced perpendicular to myofibril [c] Same as Table 1

Table 4. Epimysial Treatment with Lactic Acid

	Untrimmed Control	Trimmed Control	Lactic Acid
pH	5.80^{a}	5.78^{a}	5.52^{b}
Hydroxyproline (mg/g)	1.89^{a}	0.78^{b}	0.99^{b}
Cooking Loss (%)	27.5^{b}	29.3^{b}	34.4^{a}
Shear Force (Newtons/g)	95^{a}	61^{c}	80^{b}
Sensory Scores[a]			
Flavor	6.8^{a}	7.2^{a}	6.8^{a}
Connective Tissue	5.7^{b}	7.0^{a}	6.0^{b}
Overall Texture	6.0^{b}	6.8^{a}	6.2^{b}

Means in a row with the same superscript are not significantly different ($p \geq 0.05$)

[a] Same as on Table 1

Table 5. Microbial Collagenases and Percentage of Soluble Hydroxyproline

Enzyme Preparation	Percent Soluble Collagen
No Enzyme Control	6.2
α-Amylase	4.0
C. histolyticum	9.6*
C. histolyticum + α-amylase	8.0
A. Iophagus	6.0
A. Iophagus + α-amylase	37.4*
Vibrio B-30	16.0*
Vibrio B-30 + α-amylase	7.4

* Significantly greater than control ($p \geq 0.05$)