EFFECT OF ELECTRICAL STIMULATION ON THERMAL SHRINKAGE TEMPERATURE OF BOVINE MUSCLE COLLAGEN

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INTRODUCTION

THE QUALITY of beef may be enhanced in many ways by prerigor electrical stimulation of the musculature. Of these, the improved tenderness seems to represent the major benefit. Such an improvement has been associated with the prevention of cold shortening (Davey et al., 1976; Chrystall and Hagyard, 1976), activation of acid proteases (Savell et al., 1977) and physical disruption and formation of contracture bands (Savell et al., 1978).

The research of Bailey and Sims (1977) demonstrated that the three types of muscle collagen (Type I, III and IV) are stabilized by lysine-derived cross-links. The authors postulated that the tension generated in the collagen fibril by heating is related to meat toughness and that the extent of this tension is determined by the thermal stability of the intermolecular cross-links. However the importance of the "residual strength" of collagen and the maintenance of muscle fiber adhesion as compared to other influences on tenderness is unknown.

This research was conducted to investigate the possibility that electrical stimulation might alter the thermal stability of collagen. Such a change would provide some insight concerning the relative importance of collagen stability to tenderness as well as explain the basis of some of the tenderization produced by the electrical stimulation process.

MATERIALS AND METHODS

MUSCLE samples of 48 cattle were used in this experiment. Half of the cattle were Hereford-Angus crossbreds and half were Charolais sired and from Angus X Milking shorthorn cows. Both breed groups were further divided into feeding groups wherein the duration of grain (high energy) feeding was variable. The four groups were grain fed for 210, 140, 70 or 0 days prior to which they received low energy diets consisting of mostly roughage for 0, 77, 153 and 230 days, respectively. All cattle were slaughtered at approximately the same age. Details of the dietary treatments are reported elsewhere (Aberle et al., 1980).

Within 45 min postmortem, one side of each carcass was electrically stimulated with a 16.5 cm long electrode (33 cm^2 surface area) placed in the tissues surrounding the tibia and one in the neck region. Twenty pulses of 2 sec duration were separated by 1 sec intervals. The output of the stimulator was an AC current of 60 cycles/sec and 480V with initial amperage of 3.5A falling to approximately 2.0A at the conclusion of stimulation.

At 24 hr postmortem, longissimus muscle samples were obtained at the 13th rib with a small cork borer. Sarcomere length determinations were made using an eyepiece micrometer to observe the myofibrils suspended in 0.08M KCl (Locker, 1960). The sarcomeres were measured on 25 myofibrillar fragments of each sample.

At 7 days postmortem, 4 steaks (2.5 cm thick) were removed from stimulated and unstimulated rib sections beginning at the 12th rib surface. The steaks were frozen at -40 C and the longissimus muscles were used later for sensory tests, Warner-Bratzler shear tests, myofibrillar fragmentation index determinations and thermal shrinkage tests on intramuscular collagen.

Three steaks from each side to be used for panel and shear evaluation were cooked by oven roasting to 70 C in a gas heated oven at 165 C as described by Cross et al. (1979). They were evaluated for tenderness on a 1-8 scale (1 = extremely tough, 8 = extremely tender) by a 15 member taste panel. The shear tests were performed twice on each of three 1.25 cm cores taken from the medial side and three from the lateral side of the muscle after overnight cooling.

The myofibrillar fragmentation index (MFI) of Culler et al. (1978) was determined on muscle cores from a frozen steak. Easily fragmented samples produce short fragments with high index values in this method.

Frozen muscle samples from which the epimysial connective tissue had been removed were subjected to the collagen isolation procedure of McClain (1969). The tissues were mixed with ground dry ice and powdered with a Waring blender after which they were then passed through sieves of size 10, 14, 16 and 25. The collagen was recovered from each sieve for later thermal analysis.

The Dupont 990 thermal analyzer with a differential scanning calorimeter cell was used to determine the thermal shrinkage temperature (Ts) of the collagen. The collagen isolates were soaked in distilled water and samples weighing approximately 10 mg were hermetically sealed in aluminum cups. They were heated at the rate of 10 C/min with an empty cup as a reference. The starting temperature was approximately 21 C and the upper temperature limit was 80 C. The instrument was calibrated with benzoic acid (m.p. 122 C). A minimum of two replicate scans were performed for each sample.

An additional 10 cattle of mixed breeding and weighing 400-500 kg were used in studies of collagen solubility of longissimus muscles from electrically stimulated and unstimulated beef sides. At 7 days postmortem a 2.5 cm steak was removed at the 12th rib surface of both carcass sides. The epimysium was trimmed from the muscle

which was then frozen in liquid nitrogen and powdered in a Waring blender. Hydroxyproline extraction was performed as described by Woessner (1961) and Hill (1966). The frozen powdered samples were heated for 70 min at 77 C in 1/4 strength Ringer's solution. The soluble and insoluble fractions were separated by centrifugation and hydrolyzed for 6 hr in an autoclave in 6N HCl. After neutralization of the solutions with 1N NaOH, hydroxyproline was assayed according to the procedure of Bergman and Loxley (1963). Collagen content of the soluble and insoluble fractions were calculated by multiplying the hydroxyproline content of each by 7.25 (Goll et al., 1963).

Data were analyzed by pair-difference analyses.

RESULTS AND DISCUSSION

THE group means showing the effects of electrical stimulation on the meat quality characteristics, palatability and associated factors in this experiment are described in a separate report (Salm et al., 1980). The following discussion deals only with the breed and treatment effects on intramuscular collagen and meat tenderness.

Table 1 shows the mean Ts of intramuscular collagen in stimulated and unstimulated muscles. A highly significant difference ($P < .002$) due to stimulation was found upon subjecting the data on all cattle to pair difference analysis. This finding is interpreted to mean that the stimulation treatment lowered the Ts of the perimysial collagen. (The presence of some endomysial collagen is possible although it seems likely that such collagen would have passed through the sieves with the muscle fiber fragments.)

The perimysium includes a significant proportion of Type III collagen, the major cross-links of which are heat stable hydroxylysino-5-keto-norleucine bonds (Bailey and Sims, 1977). Since the electrical stimulation reduced the heat stability of the collagen, the possibility of a treatment induced disruption of some of these cross-links seems feasible.

When the Ts of collagen from the two breed groups were compared, the electrical stimulation seemed to be equally effective in both groups (table 1). On the other hand, the duration of the feeding period influenced the extent to which the electrical stimulation lowered the Ts of the collagen. The linear association with time on feed is readily apparent in the data summarizing differences between control and stimulated sides (table 1). The reduction of collagen Ts caused by electrical stimulation was minimal in cattle fed the longest time (210 da).

The measures of tenderness (panel score and Warner-Bratzler shear) show that the electrical stimulation improved this attribute but the improvement was greater in cattle that were not fed the high energy diets for extended periods of time (table 2). Since the muscle sarcomere length and the MFI were unaffected by electrical stimulation in any group, the interaction of electrical stimulation tenderization with time on feed appears to be independent of contraction state or physical integrity of the muscle fiber proteins.

Electrical stimulation did not affect the solubility properties of intramuscular collagen (table 3). This result seems inconsistent with the lowered Ts of collagen after electrical stimulation. However it is possible that the measure of solubility based on hydroxyproline analysis is an imprecise indicator of collagen cross-linking as compared to Ts analysis.

The degree to which small changes in the thermal stability of collagen could influence tenderness is largely unknown. Assuming that the Ts reflects, in part, the degree of intermolecular cross-linking of the collagen, one might conclude from the Ts of collagen in unstimulated muscles (table 1) that the absence of a grain feeding period favors cross-link formation. Hence the greater reduction of Ts by electrical stimulation in that group may indicate greater diminution of cross-linking by the electrical treatment.

Meat toughness may result, in part, from tension generated in the muscle by the collagen during thermal contraction (Bailey and Sims, 1977). The development of tension in collagen fibers surrounding the muscle fibers could favor ionic, hydrophobic or hydrogen bonds of denatured myofibrillar proteins and further toughening of the meat. Some of the effects of electrical stimulation could therefore be the result of subtle changes in degree of collagen cross-linking reflected as small reductions in thermal stability but as substantial improvements in meat tenderness.

SUMMARY

AN investigation was conducted to determine the effect of beef carcass electrical stimulation on the thermal stability of intramuscular collagen. Differential scanning calorimetric determinations of thermal shrinkage temperature revealed that stimulation lowered the shrinkage temperature of collagen by an average of 0.6 C in all cattle studied. No breed differences were found but the extent of the reduction of collagen shrinkage temperature was greater in animals which did not receive high grain diets or received only a short period of grain feeding than in those fed grain for up to 210 days. Added significance is provided by the fact that panel tenderness and Warner-Bratzler shear tests showed that stimulation-induced tenderization was also greater in animals fed no grain or fed grain for a short time. No evidence of stimulation effects on myofibrillar proteins was observed from data on sarcomere length or myofibrillar fragmentation index. The reduction of thermal stability of bovine intramuscular collagen by electrical stimulation may result from diminution of collagen cross-linking.

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TABLE 1 - THERMAL SHRINKAGE TEMPERATURE OF BOVINE LONGISSIMUS INTRAMUSCULAR COLLAGEN, °C.[a]

Group	N	Mean ± S.E. Control	Mean ± S.E. Stimulated	Mean difference[b]	P
Overall	48	60.0 ± 0.2	59.4 ± 0.2	0.6	.002
Breed					
Hereford-Angus crossbred	24	60.0 ± 0.2	59.4 ± 0.2	0.6	.020
Charolais crossbred	24	60.0 ± 0.3	59.4 ± 0.2	0.6	.036
Feeding duration, da					
210	12	59.8 ± 0.4	59.6 ± 0.3	0.2	.620
140	12	59.8 ± 0.2	59.3 ± 0.2	0.5	.050
70	12	59.9 ± 0.4	59.1 ± 0.3	0.8	.073
0	12	60.6 ± 0.4	59.6 ± 0.4	1.0	.030

[a]From Judge et al. (1980)

[b]Control-stimulated

TABLE 2 - DIFFERENCES (CONTROL-STIMULATED) IN LONGISSIMUS MUSCLE PROPERTIES BETWEEN ELECTRICALLY STIMULATED AND UNSTIMULATED BEEF SIDES.[a]

Group	N	Panel tenderness[bc]	Shear,[c] kg/1.25 cm	MFI[de]	Sarcomere[e] length, µM
Overall	48	-.88**	0.78**	-1.09	-.01
Breed					
Hereford-Angus crossbred	24	-1.00**	1.03**	-.94	-.02
Charolais crossbred	24	-.76**	0.53**	-1.25	0.00
Feeding duration, da					
210	12	-.38	0.45	-1.08	0.03
140	12	-.62	0.48	-1.46	-.04
70	12	-.90*	0.51**	-2.13	-.01
0	12	-1.63**	1.69**	0.29	-.01

[a]Calculated from the data of Salm et al. (1980).

[b]1 = extremely tough, 8 = extremely tender

[c]7 days postmortem

[d]Myofibrillar fragmentation index

[e]24 hr postmortem

**P < .01

*P < .05

TABLE 3 - TOTAL INTRAMUSCULAR COLLAGEN AND COLLAGEN SOLUBILITY OF BOVINE LONGISSIMUS MUSCLE[ab]

	N	Control	Stimulated	P
Total collagen, mg/g	10	3.44 ± 0.14	3.51 ± 0.14	.69
Soluble collagen, %	10	15.44 ± 0.91	14.85 ± 1.28	.42

[a]Mean ± standard error

[b]From Judge et al. (1980).