# CHEMICAL AND PHYSICAL EFFECTS OF TWO COLLAGENOLYTIC ADJUNCTS ON RESTRUCTURED BEEF

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## SUMMARY

Electron microscopy and Instron measurements were used to evaluate the effects of an *Achromobacter iophagus* culture and splenic pulp treatments on the structural and textural properties of flaked and restructured beef steaks. Both treatments improved the textural characteristics of the product when conditioned at 35°C. Electron microscopy studies revealed that the bacterial culture treatment caused a greater effect on the connective tissue elements, with a degradation of the endomysial sheath and the sarcolemma. Treatment with splenic pulp produced an overall excessive disruption, especially at the Z-lines, with little definition of the A and I bands.

## INTRODUCTION

Tenderness is an important characteristic that renders muscle foods acceptable to consumers. Collagen has been implicated in providing the "back-ground" toughness of meat (Bailey 1972). It is the main protein in connective tissue which forms fibers of high tensile strength. Particle reduction and accompanying restructuring technology enables the use of lower priced cuts of meat by mechanically breaking down connective tissue. However, the incorporation of different cuts of meat with varying amounts of connective tissue into the same product may reduce textural uniformity.

Collagenases selectively degrade connective tissue elements and thus have the potential to improve the acceptability and value of beef. Postmortem injection of bacterial collagenases into muscle (Bernal and Stanley 1986; Elkhalifa and Marriott 1988) or blending with a restructured beef product (Cronlund and Woychik 1987) causes an increase in the collagen solubility and reduction in thermal stability. Catheptic enzymes of bovine spleen have been shown to degrade myofibrillar Z-bands and the sarcolemma (Robbins et al. 1979; Cohen et al. 1982) as well as collagen (Elkhalifa and Marriott 1988).

## OBJECTIVES

This study is a continuation of our previous work on the effect of a bacterial culture and splenic pulp treatments on the collagen of restructured beef. Our objectives were to determine the shear force and to investigate the structural changes occurring within collagen and muscle fibers.

## MATERIALS AND METHODS

### Sample preparation and treatment

Postmortem muscle samples were removed from the Longissimus dorsi (LD) and shanks of U.S. Choice steer carcasses to represent low collagen (LC) and high collagen (HC), respectively. Epimysium was not removed from samples that were flaked with an Urschel Comitrol (Model 3600) using a head opening size of6.1 x 17 mm. The flaked HC particles were divided into three portions containing 1.0% NaCl and 0.25% sodium triphosphate (STP) with only one portion having 3.0% splenic pulp added. Each portion was mixed in a CSE Mixer (Model No, CDB 0615) for 10 min. The three portions were assigned to one of the following treatments: (a) HC-control; (b) HC-bacterial culture treated; (c) HC-3% (W/W) spleen treated. Samples from the LD muscle were flaked and mixed with 1.0% NaCl and 0.25% STP in a similar manner as the LC control, These samples were formed into 12.5 mm thick steaks which were wrapped in wax coated freezer paper and stored at -20°C prior to evaluation.

### Bacterial growth and injection of restructured steaks

A technique described by Keil-Dlouha et al. (1976) was used for the routine maintenance and growth of *Achromobacter iophagus* which was purchased from N.C.1.M.B., Ltd. Bacterial cultures were aerated overnight in nutrient-broth, inoculated into the culture medium (1:10 v/v), and incubated in a water bath equipped with a shaker (250 rpm) at 30°C for 5 hr to facilitate aeration. A final concentration of 2.5% peptic hydrolysate of collagen was added as a collagenase inducer to the growing culture to permit growth for an additional 6 hr to produce the collagenase enzyme.

A 6.0 (v/w) bacterial culture was injected into HC-restructured beef samples which were divided into two groups. One group was conditioned at 35°C for 3 hr and then stored at 4°C for 7 days. The other group was stored at 4°Cfor 7 days. HC control samples were injected with sterile buffer and then subjected to similar storage conditions.

### Instrumental texture analysis

The eighteen steaks used per treatment were placed into ziplock plastic bags, cooked in a water bath to an internal temperature of 68°C, and cooled to room temperature prior to cutting into samples for shear evaluation. Measurements of typical shear forces were made using the Instron Universal Testing Machine (Model 1123). Each steak was divided into 3.5 x 2.5 x 1.5 cm subsamples to give a total of 36 measurements/ treatments. A crosshead speed of 100 mm/min and a chart speed of 200 mm/min were used. Peak forces (newtons) and peak force per unit volume (newtons/$cm^3$) were determined.

### Isolation of connective tissue

Collagen from 20g minced samples was isolated according to the procedure of Fujii and Morota (1982). The collagen was washed briefly with 2% (w/v) sodium dodecyl sulfate (SDS), as described by Laurent et al. (1981), and used for electron microscopy studies.

### Scanning electron microscopy (SEM)

Approximately 1 cm particles of muscle and connective tissue were cut from each sample and fixed in 2% glutaraldehyde and 2% paraformaldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) overnight at 4°C. The fixed samples were reduced to 0.5 cm and rinsed three times (15 min each) in sodium cacodylate buffer at a pH of 7.2. The samples were washed three times in distilled water (5 min each) and dehydrated in a graded ethyl

alcohol series. Using acetone as the transition fluid, the samples were critical point dried using liquid $CO_2$ in a Ladd critical point drier. The dried samples were mounted on aluminum stubs with conductive silver paint, sputter coated with 20 nm gold-palladium in an Anatech Hummer X, examined by a Philips 505 Scanning Electron Microscope operated at 30 KV, and recorded on Polaroid-Type 55 P/N film.

**Transmission electron microscopy (TEM)**

Muscle and isolated connective tissue from each treatment were fixed in 2% glutaraldehyde and 2% paraformaldehyde in 0.1 M sodium cacodylate buffer as previously described. Then came three (15 min each) rinses in cacodylate buffer, post fixation in 2% osmium tetroxide in 0.1 M cacodylate buffer (pH 7.2) for 1 hr at 24°C, three buffer rinses (15 min each) and dehydration in a graded series of ethanol. After transition through acetone, the samples were embedded in a low viscosity medium and polymerized overnight at 70°C.

One micron sections were cut, mounted, stained with 0.5% toluidine blue and examined by light microscopy. Longitudinally-oriented areas were identified and the blocks were further trimmed and cut for electron microscopy. Thin sections were stained for 30 min with 2% uranyl acetate in 50% alcohol followed by 5 min in Reynolds lead citrate with subsequent examination using a Zeiss EM-10C transmission electron microscope operated at 60 KV.

Table 1. Comparative mean shear force values of cooked control and treated restructured beef steaks [1]

| Samples | Shear Stress (Newtons/cm$^3$) 4°C | 35°C |
|---|---|---|
| Low Collagen-control | 52.8 ± 6.86$^{Ca}$ | 40.0 + 7.12$^{Cb}$ |
| High Collagen-control | 70.5 ± 9.84$^{ABb}$ | 81.4 + 20.10$^{Aa}$ |
| High Collagen-bacteria | 74,7 ± 10.06$^{Aa}$ | 69.9 + 13.06$^{Ba}$ |
| High Collagen-3% splenic pulp | 64.3 ± 13.54$^{Ba}$ | 64.5 + 8.30$^{Ba}$ |

1 Restructured steaks were either stored at 4°C for 7 days, or conditioned at 35°C for 3 hr and then stored at 4°C for 7 days.
$^{A,B,C}$ Mean values in the same column with identical upper case superscripts are not different (P > 0.05).
$^{a,b}$ Mean values in the same row with identical lower case superscripts are not different (P > 0.05).

**Statistical analyses**

All data were subjected to analysis of variance (SAS 1982) and Tukey's HSD technique (Ott 1984) for multiple comparisons ( = 0.05) was incorporated.

## RESULTS AND DISCUSSION

**Instrumental texture analysis**

The instrumental shear measurements (Table 1) revealed that the LC-control samples required less (P < 0.05) shear force than any of the HC samples. Shear force values of the HC-control treatments at 4°C were not different (P > 0.05) from the bacteria and splenic pulp treated samples, but required less (P.05) force than the samples treated with bacteria. The shear force values for the HC-control samples at 35°C were higher (P < 0.05) than for the bacterial culture and spleen treated samples; however, there was no significant difference between the bacteria and spleen treated samples. Both the bacterial culture and splenic pulp treated samples had lower standard deviation values than the control, suggesting more uniformity among the treated samples. The reduction in shear force of the bacteria treated samples (35°C) suggests selective degradation by a collagenase enzyme in the bacterial culture while treatment with splenic pulp exhibited muscle structure breakdown by lyosomal enzymes.

**Scanning electron microscopy (SEM)**

SEM results of the muscle surface of untreated and treated samples revealed that the HC-samples treated with splenic pulp and the bacterial culture and conditioned at 35°C contained aggregated and tight entanglements of collagen fibers within the muscle tissue. Collagen fibres appeared beaded on the surface and the sarcolemma was degraded exposing the underlying myofibrils. These ultrastructural changes were not observed in the HC-samples treated with bacteria at 4°C which showed structural characteristics similar to the HC-control. Samples treated with splenic pulp exhibited muscle fibres that were more degraded and disorganized than those of the HC-control while the collagen fibres appeared somewhat fragmented and more loosely aggregated.

The LC-control samples contained thin collagen fibres which appeared loosely aggregated and structurally disorganised. Collagen of the HC-control samples appeared as dense sheets of long unfragmented fibres and coarser than those of the LC-control which is in contrast to the HC-treated with bacterial culture at 35°C that appeared to contain rather loose separated fibres and fibre bundles no longer in compact dense sheets. A similar arrangement of loose collagen fibres was also observed in the bacteria-treated HC at 4°C and splenic pulp treated samples. The bacteria treated HC-samples exhibited some areas of loosely packed sheets of collagen and, like the splenic pulp treated samples, contained disorganisation and loss of integrity of the fibre bundles.

**Transmission electron microscopy (TEM)**

Restructuring produced irregularities in the orientation of the myofibres. The sarcolemma and endomysium of the LC-samples sustained some degradation while the endomysial sheath of the HC-control samples appeared intact. The 35°C bacterial culture treated samples reflected excessive degradation of the endomysium and sarcolemma and a slight degradation of the myofibrils. The splenic pulp treated samples experienced considerable fibre disruption and the Z-lines and A and I bands were not well defined.

The LC-control samples had intact collagen fibrils with some separation into protofilaments while the collagen fibrils of HC-control samples were intact and tightly packed. The bacterial culture treatment (35°C) resulted in amorphous regions along a single fibril. Treatment with the bacterial culture at 4°C or splenic pulp did not result in amorphous regions. These results revealed that treated samples have large proportions of small diameter collagen fibres and predominantly short segments of normally banded collagen. Structural differences between control and treated samples were due to the

degradation of the endomysial sheath, myofibrils and sarcolemma.

## CONCLUSIONS

Results suggest that the bacterial culture treatment at 35°C caused greater effect on the connective tissue elements and the sarcolemma than the splenic pulp treatment which produced excessive structural changes in the myofibrils. It is reasonable to expect both treatments to contribute as exogeneous meat tenderisers, to facilitate the use of less costly cuts in restructured products at a saving.

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