Food Structure

Volume 7 | Number 2 Article 3

1988

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E. A. Elkhalifa

N. G. Marriot

R. L. Grayson

P. P. Graham

S. K. Perkins

FOOD MICROSTRUCTURE, Vol. 7 (1988), pp. 137-145
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

0730-5419/88$3.00+.00

ULTRASTRUCTURAL AND TEXTURAL PROPERTIES OF RESTRUCTURED BEEF TREATED WITH A BACTERIAL CULTURE AND SPLENIC PULP

E.A. Elkhalifa[1], N.G. Marriott[1]*, R.L. Grayson[2], P.P. Graham[1] and S.K. Perkins[2]

[1]Department of Food Science and Technology
and
[2]Electron Microscopy Laboratory
Virginia Polytechnic Institute and State University
Blacksburg, VA 24061

Abstract

Scanning electron microscopy (SEM), transmission electron microscopy (TEM) and Instron measurements were used to evaluate the effects of an Achromobacter iophagus culture (BC) and splenic pulp (SP) 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 than SP on the connective tissue elements, with a degradation of the endomysial sheath and sarcolemma. Treatment with splenic pulp produced an overall excessive disruption at the Z-lines with little definition of the A-bands.

Initial paper received April 18, 1988
Manuscript received September 07, 1988
Direct inquiries to N.G. Marriott
Telephone number: 703 961 7640

Key Words: Bacterial culture, splenic pulp, restructured beef, Instron, electron microscopy, collagen, endomysium, sarcolemma, myofibrils.

*Person to contact: N.G. Marriott
Phone number 703 961-7640

Introduction

Tenderness is one of the most important characteristics that render restructured products acceptable to consumers. Collagen has been implicated in providing the so-called "background" toughness of meat (Bailey, 1972). Connective tissue fibers can be partially broken down by flake-cutting. This particle reduction procedure and accompanying restructuring technology enables the use of lower priced cuts of meat. 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 (Eino and Stanley, 1973). Postmortem injection of bacterial collagenases into muscle (Bernal and Stanley, 1986) or blending with a restructured beef product (Cronlund and Woychik, 1987) caused an increase in the collagen solubility and reduction in thermal stability. In a recent study*, Elkhalifa and Marriott (Submitted Manuscript, 1988) found similar results by injecting a bacterial culture into restructured beef steaks. Catheptic enzymes of bovine spleen have also been shown to degrade myofibrillar Z-bands and sarcolemma (Robbins and Cohen, 1976; Cohen et al., 1982) as well as collagen (Etherington, 1976; Elkhalifa and Marriott, submitted manuscript, 1988*) and they improved the textural uniformity of pre-cooked, freeze-dried meat (Cohen et al., 1979). The objectives of this study were to observe the effect of bacterial culture and splenic pulp treatments on the collagen of restructured beef as determined by the shear force and structural changes occurring within collagen and muscle fibers.

Materials and Methods

Sample preparation and treatment

Muscle samples were obtained from animals slaughtered at the Virginia Polytechnic Institute and State University Meat Science Laboratory. Postmortem muscle samples were removed from the longissimus dorsi (LD) to represent a low collagen (LC) control treatment and the extensor carpi radialis, flexor carpi radialis, flexor capri ulnaris, superficial digital flexor and deep digital flexor muscles to represent high collagen (HC) muscles of U.S. Choice steer carcasses that were stored at 2°C for 48 hr postmortem. Epimysium was not removed and samples were cut into 3.5 x 3.5 cm pieces which were frozen at -20°C and later tempered to -4°C before flaking. The

tempered samples were flaked with an Urschel Comitrol (Model 3600) using a head opening size of 6.1 x 17 mm. The flaked HC particles were formulated to contain 1.0% NaCl and 0.25% sodium tripolyphosphate (STP) through blending in a CSE Mixer (Model No. CDB 0615) for 10 min. Three portions were assigned to one of the following treatments: (a) HC-control; (b) HC-bacterial culture treated; (c) HC-3% (W/W) splenic pulp (SP). The SP samples were divided into two groups. One group was conditioned at 35°C for 3 h and stored at 4°C for 7 days. The other group was stored at 4°C for 7 days. Both treatments were wrapped and stored in waxed freezer paper. Splenic pulp was prepared by separating the connective tissue from bovine spleen. Samples from the LD products were flaked and formulated with 1.0% NaCl and 0.25% STP in a similar manner as the LC control.

The mixed meat samples from each treatment group were stuffed into 110 mm diameter casings. The stuffed 4.2 kg logs were frozen to -20°C and then tempered to -4°C over a 16 h period. The tempered meat logs were pressed in a Ross press (Superform 720) at a setting of 37 kg/cm^2 with 2 sec dwell time into the shape of a ribeye, and sliced with a Hobart (Model 512) slicer to produce 70 g, 12.5 mm thick steaks which were then wrapped in wax coated freezer paper and stored at -20°C until testing.

Bacterial growth and injection of restructured steaks

The collagenase producing strain of Achromobacter iophagus was purchased as a dried culture from the National Collections of Industrial and Marine Bacteria, Ltd., Aberdeen, Scotland and cultured by the technique of Keil-Dlouha et al. (1976) in 2.5% casamino acids in 0.1M Tris-HCl buffer (pH 7.6) plus 0.4 M NaCl and 2 mM $CaCl_2$. The procedure was to aerate the bacteria overnight in nutrient-broth, inoculate into the culture medium (1:10 v/v), and incubate in a water bath equipped with a shaker (250 rpm) at 30°C for 5 h to facilitate aeration. A final concentration of 2.5% peptic hydrolysate of collagen was added as a collagenase inducer to the growing culture. The insoluble collagen from bovine Achilles tendon (type 1, No. 9879) was obtained from Sigma Chemical Co. (St. Louis, MO). The growth was continued for an additional 6 h for a total overall growth period of 28 h to allow production of the collagenase enzyme.

The bacterial culture (BC) was injected with a single needle containing multiple openings for uniform distribution into HC-restructured beef samples at a level of 6.0% (v/w). Samples were divided into two groups. One group was conditioned at 35°C for 3 h, wrapped in waxed freezer paper, and then stored at 4°C for 7 days. The other group was not conditioned but was wrapped in waxed freezer paper and stored at 4°C for 7 days. HC control samples were injected with sterile buffer and then subjected to storage conditions similar to those for the experimental products.

Instrumental texture analysis

In this study, eighteen steaks were used per treatment. Steaks (12.5 mm thick) were placed into Ziplock plastic bags and cooked in a water bath to an internal temperature of 68°C. After the end-point temperature was achieved, the steaks were cooled to 25°C prior to cutting into samples for shear evaluation. Measurements of 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/treatment. A crosshead speed of 100 mm/min and a chart speed of 200 mm/min were used. Peak force (newtons) and peak force per unit volume (newtons/cm^3) were determined from the recorded curve.

All data were subjected to analysis of variance (SAS User Guide, 1982). Tukey's HSD technique for multiple comparisions with the F-test at the 5% level of significance (Ott, 1984) was incorporated.

Isolation of connective tissue

Minced muscle samples (20 g) from restructured steaks were homogenized in a Brinkman Polytron and collagen was isolated according to the procedure of Fujii and Murota (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^3 particles of muscle and connective tissue were cut from each sample and fixed in 2% glutaraldehyde-paraformaldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) overnight at 4°C. The fixed samples were reduced in size to 0.5 cm^3 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 ethyl alcohol 15, 25, 40, 50, 60, 70, 80, 90, 100, 100, 100 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 and examined in a Philips 505 Scanning Electron Microscope operated at 30 kV. Micrographs were recorded on Polaroid-Type 55 P/N film.

Transmission electron microscopy (TEM)

Samples of muscle and isolated connective tissue from each treatment were fixed in 2% glutaraldehyde-paraformaldehyde in 0.1 M sodium cacodylate buffer as described above. This procedure was followed by 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 h at 24°C, three buffer rinses (15 min each) and dehydration in a graded series of ethanol as previously described. After transition through acetone, samples were embedded in a low viscosity embedding medium (Spurr, 1969) and polymerized overnight at 70°C.

One micron sections were cut on a glass knife, 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 (Reynolds, 1963) with subsequent examination using a Zeiss EM-10C transmission electron microscope operated at 60 kV.

Results

Instrumental texture analysis

The instrumental shear measurements are presented in Table 1. The LC-control samples required less (P less than 0.05) shear force than the HC samples regardless of the treatment. Shear force values

of the HC-control samples at 4°C were not different (P greater than 0.05) from the bacteria and splenic pulp treated samples. The shear force value for the HC-control samples at 35°C was higher (P less than 0.05) than for the bacterial culture and spleen treated samples; however, there was no significant difference between the BC and SP treated samples. Both the BC and SP samples had lower standard errors than the control which suggests more uniformity among the treated samples.

Table 1. Mean shear force values for cooked control and texture-modified restructured beef steaks[1]

Samples	Shear Stress (Newtons/cm^3)	
	4°C[2]	35°C[2]
	$\bar{X}$ SE	$\bar{X}$ SE
LC*-control	52.8 ± 6.86^{Ca}	40.0 ± 7.12^{Cb}
HC**-control	70.5 ± 9.84^{ABb}	81.4 ± 20.1^{Aa}
HC + A. iophagus culture[3]	74.7 ± 10.06^{Aa}	69.9 ± 13.06^{Ba}
HC + splenic pulp[4]	64.3 ± 13.54^{Ba}	64.5 ± 8.30^{Ba}

[1] N=18 steaks per treatment.

[2] Products were stored at 4°C for 7 days, or conditioned at 35°C for 3 h and then stored at 4°C for 7 days.

[3] 6% (V/W) bacterial culture injected into steaks.

[4] 3% splenic pulp added before forming steaks.

A,B,C Mean values in the same column with identical upper case superscripts are not different (P greater than 0.05).

a,b Mean values in the same row with identical lower case superscripts are not different (P greater than 0.05).

* LC=low collagen.

** HC=high collagen.

Scanning electron Microscopy (SEM)

Results and discussion are slightly abbreviated by discussion of only the SP samples that were stored at 4°C, since the data in Table 1 were not different (P greater than 0.05) between the two temperatures. Fig. 1 illustrates the SEM results of the muscle surface of untreated and treated restructured muscle samples. The LC-control (Fig. 1A) which possessed 4.7 mg/g tissue of collagen, contains few collagen fibers which allows the muscle fiber surface to be observed directly although the muscle fiber observed is a small part of the micrographic field shown. The HC-control (Fig. 1B) which had 20.2 mg/g tissue of collagen, is more heavily covered by collagen fibers than the other treatments. The structure of the HC-muscle tissue appeared to be looser and less compact than the LC-control. Representative HC-samples injected with the bacterial culture and conditioned at 35°C (Fig. 1C) showed major effects of this treatment by exhibiting aggregated and tight entanglements of randomly selected collagen fibers within the muscle tissue. In addition, Fig. 1C shows an area of muscle tissue where collagen fibers appear beaded on the surface and the sarcolemma has been degraded exposing the underlying myofibrils. These ultrastructural changes were not observed in the HC-samples treated with bacteria at 4°C (Fig. 1D), which showed structural characteristics similar to the HC-control. Samples treated with splenic pulp revealed some degradation of the sarcolemma exposing the underlying myofibrils (Fig. 1E). The muscle fibers appeared more degraded and disorganized than those of the HC-control. In addition, collagen fibers appeared somewhat fragmented and more loosely aggregated.

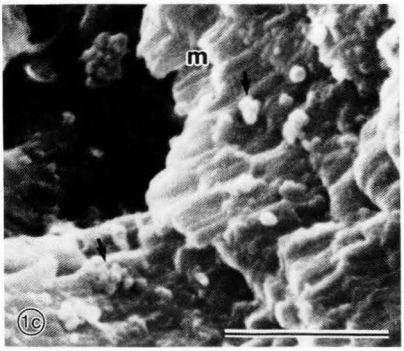
SEM was also used to study the morphology of isolated collagen fibers from the control and treated samples (Fig. 2). The LC-control samples (Fig. 2A) contained thin collagen fibers which appeared loosely aggregated and structurally disorganized. Collagen of the HC-control samples appeared as dense sheets of long unfragmented fibers (Fig. 2B). Because of their tight association, the collagen fibers appeared coarser than those of the LC-control. This is in striking contrast to the HC-treated with bacterial culture at 35°C which (Fig. 2C) appears to contain rather loose separated fibers and fiber bundles no longer in compact dense sheets. A similar arrangement of loose collagen fibers was also observed in the bacteria-treated HC at 4°C (Fig. 2D) and splenic pulp treated (Fig. 2E) samples. However, Fig. 2D does show some areas of loosely packed sheets of collagen and, like Fig. 2E, there is disorganization and loss of integrity of the fiber bundles. These observations support the differences in the Instron values of the LC- and HC-control samples.

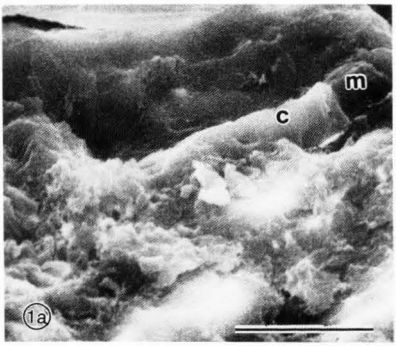
Transmission electron microscopy (TEM)

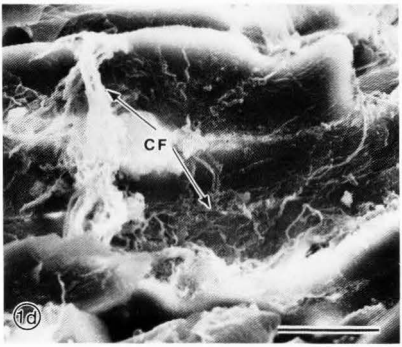
Transmission electron micrographs representative of the control and treated restructured muscle samples are shown in Fig. 3. As seen in Fig. 3A and 3B, restructuring produced irregularities in the orientation of the myofibers. For the LC-control sample (Fig. 3A), the sarcolemma and the endomysium have some degradation. The endomysial sheath of the HC-control sample (Fig. 3B) appears to remain intact. However, the 35°C BC treated samples (Fig. 3C) reflect excessive degradation of the endomysium and sarcolemma. A slight degradation of the myofibrils occurred in certain areas. As illustrated in restructured samples treated with bacteria (Fig. 3D), some degradation occurred in the endomysium as well as the myofibrils. The SP samples (Fig. 3E) reflect considerable overall fiber disruption. The Z-lines and A and I bands are not well defined. Less degradation was evident in the endomysium and sarcolemma components than in the HC-controls (Fig. 3B).

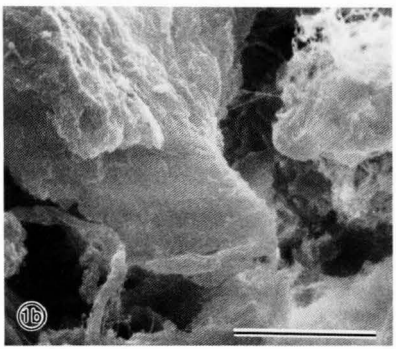
The TEM micrographs of isolated collagen from control and treated samples are shown in Fig. 4. Collagen fibrils of LC-control samples have intact fibrils with separation of some fibrils into protofilaments (Fig. 4A). In Fig. 4B, collagen fibrils of HC-control samples are intact and tightly packed. The

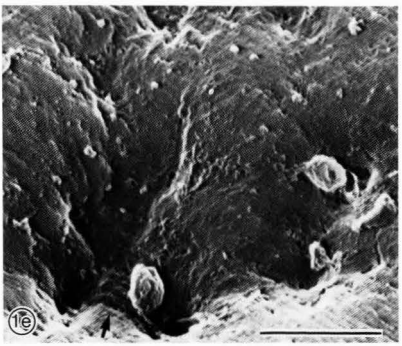
Fig. 1. SEM Micrographs of: (A) A low collagen (LC-control) restructured control sample showing muscle tissue and associated collagen (C). Note the lack of individual collagen fibers over the surface of the muscle fiber (M). (B) A high collagen (HC-control) restructured sample. The surface of muscle fibers is thickly covered by collagen sheets. (C) A high collagen sample (HC-bacteria) treated with bacteria at 35°C. An area is shown in which the collagen fibers beaded on the surface (arrows) and underlying muscle fibers (M) are exposed. (D) A high collagen sample treated with bacteria at 4°C. Note the presence of collagen fibers (CF) on the surface of the tissue and the structural similarity to the control shown in Figure 2B. (E) A high collagen sample treated with splenic pulp and stored at 4°C. An area is shown in which the sarcolemma appears to have lost some of its integrity resulting in the exposure of what may be underlying myofibrils (arrow).





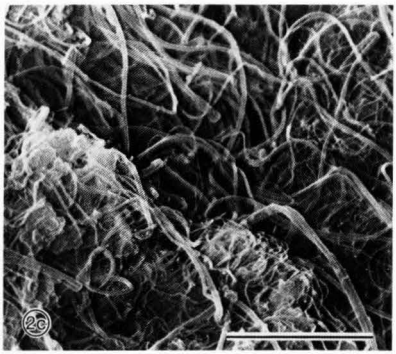


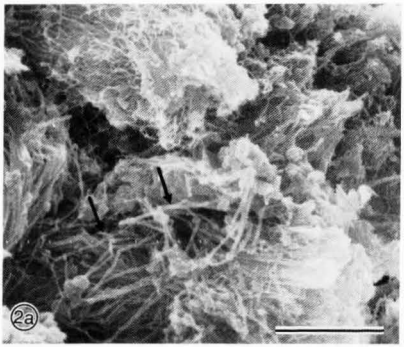


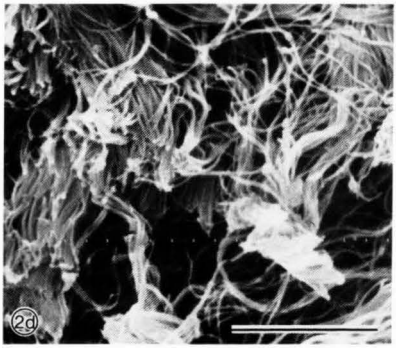


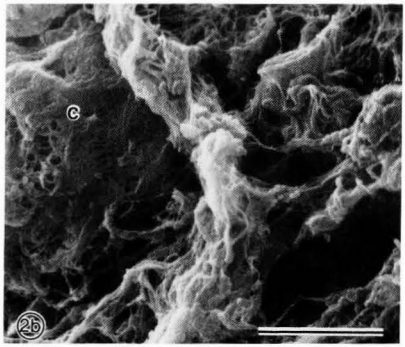
Bars = 50 μm (for A, D and E), 10 μm (for B) and 5 μm (for C).

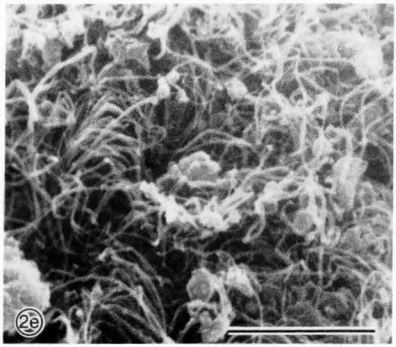
Fig. 2. SEM micrographs of collagen fibers isolated from: (A) LC restructured control sample. The sample contains a high proportion of thin collagen fibers (arrows) which appear disorganized and loosely arranged. (B) HC restructured control sample. Collagen appears as dense sheets (C) composed of highly compacted fibers. (C) HC bacteria sample treated with bacteria at 35°C. Note the loose arrangement and separation of both thick and thin collagen fibers resulting from the degradative action. (D) HC sample treated at 4°C. Partial loosening of collagen fibers implies less bacterial degradation at 4°C than at 35°C (Figure 2C). (E) HC sample treated with splenic pulp and stored at 4°C. The loose arrangement of the collagen fibers appears similar to that seen in the samples treated with bacteria at 4°C (Figure 2D).





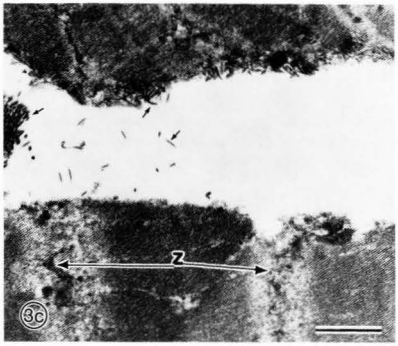


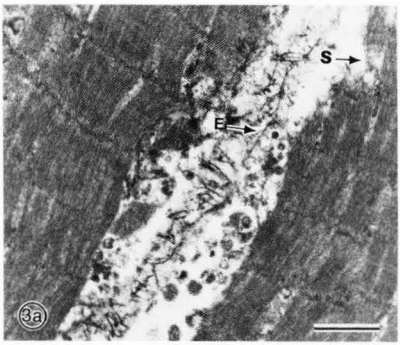


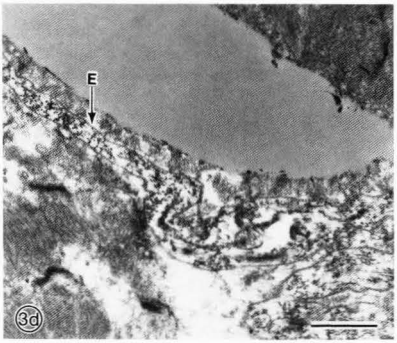


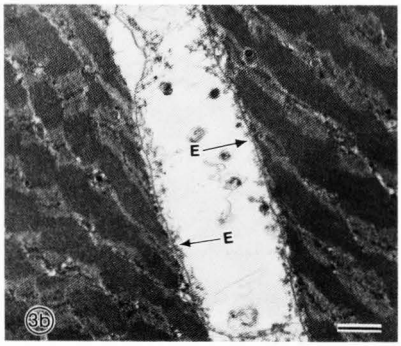
Bars = 10 μm (for A, B and E); = 5 μm (for C and D).

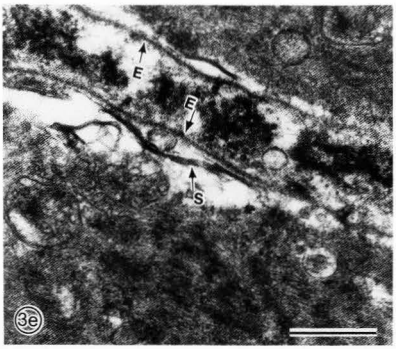
Fig. 3. TEM micrographs of: (A) Low collagen restructured control sample showing small remnants of the sarcolemma (S) and endomysium (E). There is some disruption of the structural organization of Z-lines and myofibrils due to the restructuring of tissue. (B) High collagen restructured control sample. In this oblique section, the endomysial sheath (E) appears to be intact. Structural disorganization of the myofibrils and associated components has resulted from restructuring or other forms of mechanical disruption of the muscle tissue. (C) High collagen restructured sample treated with bacteria at 35°C showing complete degradation of the endomysium, sarcolemma and Z-line (Z) configuration of the myofibrils. The space between the myofibers contains collagen remnants (small arrows). (D) High collagen restructured sample treated with bacteria at 4°C. Notice slightly less degradation of endomysium (E), myofibrils and Z-lines as compared to Fig. 3C. Cross-sections of collagen fibers occupy the space external to the endomysia. (E) High collagen restructured sample treated with splenic pulp and stored at 4°C. Z-lines and A and I bands are no longer apparent due to excessive disruption of the myofibrillar structure caused by the action of hydrolytic enzymes. Remnants of the endomysium (E) and sarcolemma (S) are present.





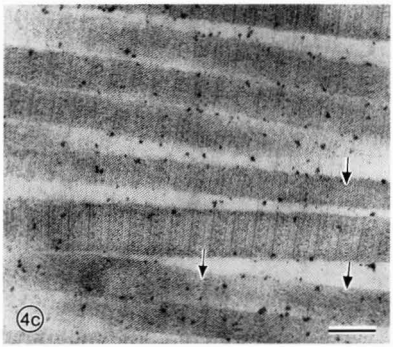


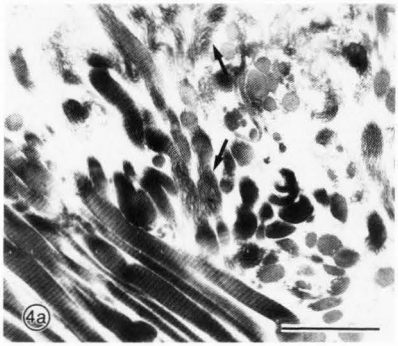


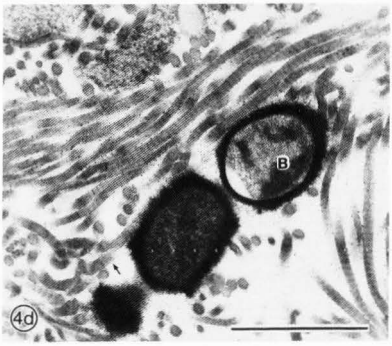


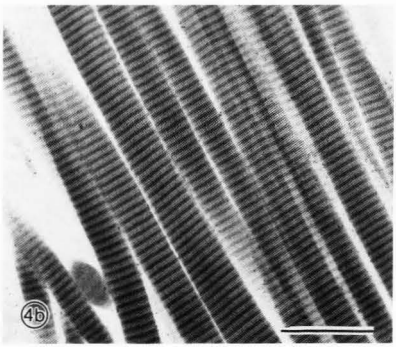
Bars = 1 μm (for A, B, C, and D) and 0.5 μm (for E).

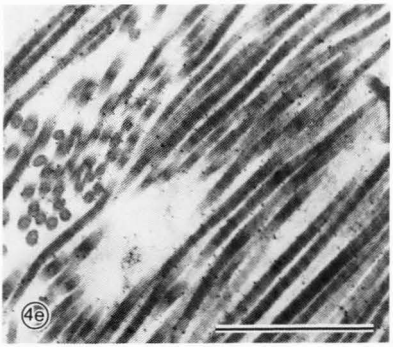
Fig. 4. TEM micrographs of collagen fibrils isolated from: (A) A low collagen restructured control sample showing intact fibrils separated with some fibrils into protofilaments (arrows). (B) A high collagen restructured control sample. The collagen fibrils appear to be intact and tightly packed. (C) A high collagen restructured sample treated with a bacterial culture at 35°C. Amorphous regions can be seen within a single fibril (arrows). (D) A high collagen restructured sample treated with a bacterial culture at 4°C. Orientation of collagen fibrils has been disrupted as evidenced by numerous cross-sections relative to the control. This may have been caused by normal factors i.e., placement in block (4B). There is some separation of fibrils into protofilaments (arrow) similar to the low collagen control (4A). The diameter of the collagen fibrils is smaller than controls. Note bacteria (B). (E) A high collagen restructured sample treated with splenic pulp and stored at 4°C. This micrograph reveals that collagen fibril diameters are less than the controls. Cross-sections of collagen can be observed as being dispersed among longitudinal segments of banded collagen.











Bars = 1 μm (for A and D), 0.5 μm (for B and E), and 0.1 μm (for C)

BC treatment (35°C) resulted in amorphous regions along a single fibril (Fig. 4C). Figures 4D and 4E suggest that treatment with the bacterial culture at 4°C or splenic pulp (Figs. 4D and 4E) did not result in amorphous regions. These observations reveal that treated samples (Figs. 4C, 4D and 4E) have large proportions of relatively small diameter collagen fibers and predominantly short segments of normally banded collagen, suggesting effects of the BC and SP treatments.

Discussion

The reduction in shear force of the bacteria treated samples (35°C) is a reflection of the selective degradation of collagen (Figs. 3C and 4D) by a collagenase enzyme in the bacterial culture. Treatment with splenic pulp at either temperature resulted in excessive disruption of the myofibrillar structure as evidenced by the degradation of Z-lines and A and I bands (Fig. 3E) which was attributable to the lysosomal enzymes in spleen. In work on the effect of catheptic enzymes from spleen, Cohen et al. (1979) found that enzyme-treated samples were more tender and more uniform in texture.

As seen in the SEM micrographs, treatment with either the bacterial culture or splenic pulp gave rise to surface structural changes of the restructured samples. Using SEM, Eino and Stanley (1973) observed similar hydrolytic changes in the connective tissue and sarcolemma when rabbit psoas muscle was incubated with a bacterial collagenase. In a study on the muscle fiber surface of restructured beef, Cohen et al. (1982) demonstrated that catheptic enzymes from splenic pulp caused degradation of the sarcolemma and exposure of the myofibrils in restructured beef. Results from this investigation support what earlier researchers have reported and add to the knowledge base of the effects of these two treatments.

Ultrastructure of muscle and connective tissue of a restructured product have not been studied specifically by TEM. The structural differences between control and treated samples seen in the TEM micrographs (Figs. 3 and 4) are the degradation of the endomysial sheath, myofibrils and the sarcolemma. The degradation of sarcolemma and endomysium due to the bacterial culture treatment has important implications. Offer (1984) suggested a mechanism for water loss from muscle during cooking that involves shrinkage of actomyosin within muscle cells to leave free fluid which can then purge out. Since the structure of the endomysium is in intimate contact with such shrunken actomyosin components in samples cooked to 77°C, it may accelerate this passive process. Light et al. (1985) suggested that the endomysium may present a barrier to transverse fracture of muscle pieces which can develop a compressive force as shrinking constricts muscle cells, thus squeezing free water out of the cut ends of the muscle in an active process and thereby contributing to increased toughness. This hypothesis may be correct since the degradation of the endomysial sheath in BC samples at 35°C may contribute to the reduction in shear force. The improvement in tenderness of HC samples from the SP is evidence of the extensive degradation of the myofibrillar structure.

In view of the results presented here, there is no doubt that there was a greater effect on the connective tissue elements and sarcolemma by the 35°C bacteria treatment than by the SP treatment which produced microstructural changes within the myofibrils. It is reasonable to expect the technology of both treatments to be quite useful as exogeneous meat tenderizers to permit the use of less costly cuts of meat at significant monetary savings.

Acknowledgments

The authors thank Dr. K. C. Diehl for assistance with the instrumental texture analysis. Assistance from Mr. Steve Phelps is also acknowledged. The authors express their appreciation to the Virginia Cattle Industry Board and the Virginia Agricultural Council for financial support. Appreciation is extended to Ross Industries, Inc. for use of the Ross Superform 720 Press and to Urschel Laboratories, Inc. for loan of the Comitrol 3600.

References

Bailey AJ (1972). The basis of meat texture. J. Sci. Food Agric. 23, 995-1007.

Bernal VM, Stanley DW (1986). Proteolysis of intramuscular connective tissue during postmortem conditioning of beef muscle. In: Proc. 32nd Europ. Meeting of Meat Res. Workers. 32, 161-164. Ghent, Belgium.

Cohen SH, Kostick JA, Robbins FM, Segars RA, Walker JE (1979). Action of spleen extracts on stored pre-cooked freeze-dried beef. J. Food Sci. 44, 1118-1120.

Cohen SH, Segars RA, Cardello A, Smith J, Robbins FM (1982). Instrumental and sensory analysis of the action of catheptic enzymes on flaked and formed beef. Food Microstruc. 1, 99-105.

Cronlund AL, Woychik JH (1987). Solubilization of collagen in restructured beef with collagenases and α-amylases. J. Food Sci. 52, 857-860.

Eino MF, Stanley DW (1973). Surface ultrastructure and tensile properties of cathepsin and collagenase treated muscle fibers. J. Food Sci. 38, 51-55.

Etherington DJ (1976). Bovine spleen cathepsin B_1 and collagenolytic cathepsin: A comparative study of the properties of the two enzymes in the degradation of native collagen. Biochem. J. 153, 199-209.

Fujii K, Murota K (1982). Isolation of skeletal muscle collagen. Anal. Biochem. 127, 449-452.

Keil-Dlouha V, Mirashi R, Keil B (1976). The induction of collagenase and neutral proteinase by their high molecular weight substrates in Achromobacter iophagus. J. Mol. Biol. 107, 293-305.

Laurent GJ, Cockerill P, McAnulty RJ, Hasings JRB (1981). A simplified method for quantitation of the relative amounts of type I and type III collagen in small tissue samples. Anal. Biochem. 113, 301-312.

Light ND, Champion AE, Voyle C, Bailey AJ (1985). The role of epimysial, perimysial and endomysial collagen in determining texture in six bovine muscles. Meat Sci. 13, 137-149.

Offer G (1984). Progress in biochemistry, physiology and structure of meat. In: Proc. 30th Europ. Meeting of Meat Res. Workers. 30, 87-94. Bristol.

Ott, L (1984). An Introduction of Statistical Methods and Data Analysis, 2nd ed. PWS Publishers, Boston.

Reynolds ES (1963). The use of lead citrate at high pH as an electron-opaque stain in electron

microscopy. J. Cell Biol. 17, 208-212.

Robbins FM, Cohen SH (1976). Effects of cathepsic enzymes from spleen on the microstructure of bovine semimembranosus muscle. J. Texture Studies 7, 137-142.

SAS User Guide (1982). Statistics. Ray AA (Ed.). SAS Inst., Inc., Cary, NC.

Spurr, AR (1969). A low viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. 26, 31-43.

Discussion with Reviewers

Reviewer 1: Please describe the handling of the carcasses postmortem.
Authors: After the U.S. Choice steers were slaughtered, they were transferred from ambient temperature (25°C) to a chill cooler maintained at 2°C. After carcass storage for 48 h, the muscles were excised as described in the materials and methods of this paper.

Reviewer I: What is the reason for including the LC samples?
Authors: The low collagen (LC) samples were studied to compare their shear force values and ultrastructural characteristics with the other treatments. Our major objective for studying the LC samples was to determine how closely the samples treated with a bacterial culture or splenic pulp would resemble the LC controls.

Reviewer I: Where did you get or how do you prepare and store splenic pulp?
Authors: The splenic pulp was obtained from the spleens obtained during slaughter of these animals. Immediately before excising the muscles for this study, the connective tissue was separated from the bovine spleens and the resulting pulp was incorporated in the formulation.

Reviewer I: What was the concentration of bacteria in the inoculum used for injection of steaks? How old was the culture? What was the concentration of enzyme? Where do you inject it? Does any run out? Are the steaks wrapped for storage?
Authors: Concentration of the bacterial suspension was determined with the spectrophotometer. The concentration was 100 ml w/OD of 4.0-5.0 at 600 nm. The suspension was injected immediately after culture development. The concentration of the enzyme was unknown because living bacterial cells were used. Multi-injection was into the interior of the steaks with a single needle containing multiple openings for uniform distribution throughout the restructured product. Although we did not weigh the samples to determine moisture loss, no exudate was observed. This observation may be attributable to the STP in the formulation. The steaks were wrapped with waxed freezer paper prior to storage.

Reviewer I: What was the collagen content of the LC versus the HC samples?
Authors: The mean collagen content of the LC samples was 4.7 mg/g tissue. The HC samples had a mean collagen content of 20.2 mg/g tissue.

Reviewer I: Is one of your conclusions that more fibrous collagen occurs in the HC control?
Authors: This answer is based on our assumption that the reviewer meant more fibrous collagen occurs in the HC control than the LC control and HC samples treated with bacterial culture or splenic pulp. Yes, we concluded that more fibrous collagen occurs in the HC control. The LC samples had less collagen as evidenced by another study that has been submitted for publication separately.* The BC and SP samples had less fibrous collagen because of collagen degradation that occurred.

Reviewer I: Are the control microscopy samples randomly selected from your cuts? In samples such as these there must be considerable variation in the "terrain" to be viewed on a sample. How extensive was your microscopy? How many samples?
Authors: The samples were selected randomly. Fifteen samples per treatment were observed.

Reviewer I: How can you tell the difference between endomysium and sarcolemma in some of your TEM micrographs?
Authors: The endomysium is thicker since it surrounds the muscle fiber. The sarcolemma is thinner and is the innermost membrane. By the morphology location and measurement of the structures, we were able to distinguish between the endomysium and sarcolemma.

Reviewer IV: What are casamino acids? Are they available from a commercial supplier or must they be prepared in the laboratory?
Authors: Casamino acids is acid hydrolyzed casein recommended for use in microbial culture media which require a completely hydrolyzed protein as a nitrogen source. Casamino acids is well suited for the preparation of "synthetic" or chemically defined media. We incorporated this compound for the maintenance and growth of the A. iophagus culture used in this study. It is available commercially through Difco in Detroit, Michigan.

D.N. Holcomb: Please provide more details of the testing fixture used in measuring the shear forces with the Instron testing machine.
Authors: A cast aluminum alloy housing was incorporated with sample space size as follows: 67 mm between smooth walls, 66 mm between slotted walls and a depth of 62 mm. Ten blunt-ended, teflon-coated blades with a width of 3 mm and depth of 70 mm are guided into the slots, spaced at 3 mm apart, by a removable cover plate which rests on the top of the cell. The blades are suspended from an aluminum alloy housing which attaches to the load cell on the Instron testing machine.

*This companion paper has been submitted for publication. Preprints are available from N. G. Marriott, 103 Food Science Building, VPI & SU, Blacksburg, VA 24061.