EFFECTS OF ORGANIC ACIDS ON THE COLLAGEN OF RESTRUCTURED BEEF

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INTRODUCTION

Restructured meat products are manufactured from the less expensive cuts. Thus, these resulting products lack tenderness when high levels of connective tissue are present because of the presence of fibrillar collagen.

Tenderness of these products can be increased through degradation of the intrinsic collagen structure to produce the desired tenderness and texture, i.e. without extensive degradation of muscle fibers (Cronlund and Woychik, 1987). Enzymatic breakdown of collagen with plant proteinases such as papain, bromelain and ficin and with bacterial collagenases has been used as a method of tenderization; (Dransfield and Etherington, 1981; Foegeding and Larick, 1987). Furthermore, the postmortem injection of bacterial collagenase into bovine muscle has been shown to produce an increase in collagen solubility (Cronlund and Woychik, 1987; Elkahalifa and Marriott, 1988) and a reduction in denaturation temperature (T_D) of collagen (Bernal and Stanley, 1986; Elkhalifa and Marriott, 1988). Collagenases from many sources have been active on isolated collagen (Bernal and Stanley, 1987; Cronlund and Woychik, 1987) but unsuccessful in a meat product (Foegeding and Larick, 1986). Catheptic enzymes of bovine spleen have been shown to degrade myofibrillar Z-bands and sarcolemma (Robbins and Cohen, 1976; Cohen et al., 1982).

One disadvantage of enzymatic tenderization of meats with plant and microbial proteases is the preferential hydrolysis of myofibrillar and sarcoplasmic proteins over stromal tissue proteins (Fogle et al., 1982). This action can result in extensive degradation of the muscle structure and undesirable textural quality.

A viable technique for the tenderizing of meat is the use of organic acids. Acetic acid has been used to marinade meat for tenderness improvement (Gault, 1984). Strange and Whiting (1988) immersed strips of Supraspinatus, Infraspinatus, and Triceps brachii muscles in 0.5 M lactic acid solution for 30 min at 23°C. They found that lactic acid decreased the shear force values of epimysial connective tissue in restructured beef steaks. Thus, the objective of this study was to determine the effect of acetic, citric and lactic acids on the tenderness of restructured beef steaks.

MATERIALS AND METHODS

Product Preparation

High collagen (HC) muscles (Extensor carpi radialis, Flexor capri radialis, Flexor capri ulnaris, Superficial digital flexor and Deep digital flexor) were removed from 20 U.S. Good steer carcasses 72 hr postmortem. Low collagen (LC) Longissimus dorsi muscles were excised from the 8th to 11th thoracic vertebrae. Particle reduction of these muscles was accomplished through a kidney plate grind. The HC muscles were separated into five equal batches that were allotted to each of the following treatments: (a) HC-unconditioned control (HUC); (b) HC conditioned control (HCC); (c) HC-acetic acid treated (A); (d) HC-citric acid treated (C); and (e) HC-lactic acid treated (L). The LC muscles were allotted to the LC-control (LCC).

The restructured beef roasts for the control samples were manufactured by mixing the ground muscle with 10% water, 1% NaCl and 0.3% sodium tripolyphosphate (STP). These samples were thoroughly mixed for 10 min, stuffed into 64.5 mm diameter casings, frozen to -20°C and tempered to -4°C for 16 hr. The restructured logs were then pressed into the desired shape using a Ross Superform 720 press, sliced to a thickness of 12.5 mm, and wrapped separately in wax coated freezer paper. The HCC samples and the other acid-treated samples (A, C and L) were conditioned at 4°C for 120 hr prior to freezer storage.

Acetic, citric and lactic acid solutions were prepared to provide 0.75% of the total weight of the restructured product. These products were then subsequently processed in the same manner as the control samples.

Collagen Solubility

Collagen solubility was determined according to the method of Hill (1966). Approximately 5 g samples were freeze-dried for 48 hr. The freeze-dried samples were then powdered by a Wiley mill through a 20 mesh sieve. Twenty-five mL of 1/4 strength Ringer's solution was added and the mixture was heated to 90°C for 1 hr in a shaking water bath. The mixture was centrifuged (400 x g for 10 min) after which the supernatant was decanted and saved for further analysis. An additional 8 mL of 1/4 strength Ringer's solution was added to the residue. The mixture was then vortexed for 10 sec and centrifuged again at 4000 x g for 10 min.

The supernatant was then combined with the first fraction. The supernatant and residue fractions were individually hydrolyzed with 50 mL 6N HCl for 20 hr at 120°C. After neutralization of the hydrolyzates with 6N NaOH using methyl red as a titrating indicator, the hydroxyproline content of each fraction was determined according to the procedure of Bergman and Loxley (1963). The hydroxyproline contents of the residue and the supernatant were multiplied by 7.25 and 7.52, respectively to yield the collagen contents. Total collagen contents of the sample was taken as the sum of the collagen contents of both residue and supernatant. Percentage soluble collagen was calculated by dividing the collagen content of the supernatant by the total collagen.

Protein Solubility

The method of Hwang et al. (1977) was used to determine the protein solubility of the different protein fractions. Twenty-five mL of precooled 0.03M phosphate buffer was added to 2 g samples. The mixture was homogenized at 40,000 rpm with a Brinkman Polytron for 5 sec at 22°C to extract the sarcoplasmic proteins. The homogenate was transferred to a centrifuge tube. The homogenizer blades were rinsed in 10 mL buffer which was added to the homogenate. The homogenate was centrifuged at 27,000 x g for 15 min at 4°C. The residue was then similarly extracted with precooled 1.1M KI in 0.1M potassium phosphate buffer to separate the myofibrillar proteins. The protein content of the different extracts was measured by the biuret method (Clarke and Switzer, 1977).

Shear Force Determination

Frozen samples from each treatment were tempered at -4°C for 16 hr with subsequent placement in Whirl-pak bags (530 mL) for cookery in a preheated water bath (80°C) to an internal temperature of 65°C. Upon reaching the desired endpoint temperature, the samples were cooled to room temperature. The restructured products were divided into approximately 3.5

x 1.5 x 1.5 cm samples. The volumes of the samples were measured using an air/comparison pyncnometer. The shear force values were determined using the Instron Universal Testing Machine (Model 1123) with a Kramer shear attachment controlled by a microcomputer. A crosshead speed and full scale load of 100 mm/min and 10,000 newtons, respectively were used. These parameters were set and maximum peak forces were acquired by a micro-computer. Shear force values were expressed in newtons/cm^3.

Differential Scanning Calorimetry

Collagen was isolated by the procedure of Fujii and Murota (1982). Twenty g of minced muscle were homogenized with a Brinkman Polytron (40,000 rpm) for 5 sec at 22°C in 5 volumes (v/w) of 10 mM Tris-maleate containing 0.1 M KCl (pH 7.2). The homogenate was then stirred rapidly for 12 hr. The fibrous material was collected by passing the homogenate through cheese cloth. This material was subsequently extracted with: (a) 20 volumes (v/w) of Hasselbach-Schneider solution (3 repetitions) and (b) 20 volumes (v/w) of 0.6 M KI-0.06 M Na_2SO_3 (2 repetitions). After the final extraction, the collagen was rinsed with distilled water, freeze-dried for 48 hr and stored desiccated at -20°C until required.

Collagen samples from each treatment were ground once through a Wiley Mill with a 20 mesh sieve. A 50 mg sample was rehydrated in 5 mL of distilled water for 24 hr at 4°C. The rehydrated sample was then allowed to pass through a glass fiber filter disc (19 mm diameter). The filtered samples (15-30 mg) were weighed in Perkin-Elmer sample cups and sealed. With a sealed empty cup serving as the reference, the samples were heated in a Perkin Elmer Differential Scanning Calorimeter (Model 4) from 25 to 100°C at a heating rate of 5° C/min. This instrument was previously calibrated with an indium standard by heating from 50 to 175°C at a heating rate of 10°C per min. The onset temperature as determined by the Perkin Elmer data station was taken as the denaturation temperature of collagen (T_D).

Color Determination

The HunterLab colorimeter (Model D 25), was used to measure the "L", "a", and "b" values of both the uncooked and cooked samples. Three readings at different locations for each sample were taken. Four of the samples were cooked according to the procedure described for Shear Force Determination.

Statistical Analyses

This entire experiment was conducted as a randomized complete block design with three replications. The data were analyzed by the General Linear Models Procedure (SAS User Guide, 1982). Treatments were compared using the multiple range test (Duncan, 1955) at the 5% level of significance.

RESULTS AND DISCUSSION

Collagen Solubility

The collagen solubilities of the acid-treated restructured beef steaks (A, C and L) were greater ($P \leq 0.05$) than the control steaks (HUC and HCC) made of high collagen beef (Table 1). Among the three acids, lactic acid was the least effective, having a collagen solutility closest to the HC controls (HUC and HCC). These data suggest that citric acid was the most effective, having a collagen solubility closest to the low collagen control (LC).

Total collagen of the acid-treated samples was greater ($P \leq 0.05$) than for the two HC controls (Table 1). The pH values of the acid-treated samples

were less ($P \leq 0.05$) than the control. As a result, protein denaturation lowered the water-holding capacity of the proteins and induced the loss of water from the samples producing unusually elevated levels of total collagen in the samples. The higher total collagen content of the acid-treated samples correspond with greater shear force values (Table 2). A trend was observed (Table 2) in which the shear force values for treatments A and L were numerically higher but not significantly ($P>0.05$) greater than treatment HCC. Treatment C had a shear force value nearly identical to treatment HCC; whereas, the shear force values for the acid-treated samples were not different ($P>0.05$) than the HC control (HCC). This treatment had a hardening effect on other muscle proteins in addition to solubilizing effect on collagen. No positive correlation between collagen solubility and shear force measurements was observed.

Protein Solubility

Organic acids did not have any effect ($P>0.05$) on the amount of sarcoplasmic protein. Yet, acid treatment resulted in a lesser amount of myofibrillar protein compared with the controls (HUC, HCC, and LC). Treatment L had the least effect on solubility. The addition of acid lowered the pH thus possibly inhibiting the degradation of muscle proteins by endogenous enzymes. This phenomena could decrease the extractability of myofibrillar protein.

Differential Scanning Calorimetry

Collagen from samples treated with acid exhibited a decrease ($P \leq 0.05$) in denaturation temperature (T_D) and the enthalpy of denaturation (ΔH) compared with the controls (treatments HUC and HCC). These results indicate that acid treatment resulted in denaturation of the collagen as indicated by the lower denaturation temperatures. The acid appeared to enhance alterations in the structural stability of the collagen, with the effect being least pronounced in samples treated with lactic acid.

Table 1. Solubility and collagen content of treatments with organic acids.

Treatment	% Collagen Solubility[a]	Total Collagen[a] (mg/g tissue)
	$\bar{X}$	$\bar{X}$
High collagen unconditioned control	20.66^{bc} (1.86)	29.96^{cd} (1.19)
High collagen conditioned control	18.23^{b} (1.93)	24.08^{c} (2.07)
Low collagen control	42.83^{e} (5.82)	4.48^{b} (0.33)
High collagen acetic acid treated	33.23^{de} (1.52)	38.32^{ef} (4.66)
High collagen citric acid treated	38.35^{ef} (1.31)	43.73^{f} (1.78)
High collagen lactic acid	27.56^{cd} (0.95)	32.59^{de} (1.54)

[a]Standard error values ranged from 0.33 to 5.82.

[bcdef] Means in the same column with identical superscripts are not different ($P>0.05$).

Color

The uncooked acid-treated samples yielded lower "a" values (Table 3). The changes in color of the uncooked

Table 2. Mean shear values of differing treatments.

Treatment	Shear Force (Newtons/cm^3)	
	$\bar{X}$	S.E.
High collagen unconditioned control	114.16[b]	10.25
High collagen conditioned control	141.20[ab]	17.16
Low collagen control	35.07[c]	0.83
High collagen acetic acid treated	180.28[a]	16.75
High collagen citric acid treated	141.61[ab]	15.67
High collagen lactic acid treated	178.58[a]	14.66

[abc] Means in the same column with identical superscripts are not different (P>0.05).

products are further reflected in the cooked samples (Table 4). Subjective visual observations indicated that uncooked acid-treated samples were brownish in appearance whereas the cooked acid-treated samples were whiter when compared with their control counterparts. The acid treatment appeared to enhance the conversion of myoglobin to metmyoglobin which has a lower color intensity. Furthermore, the lowered pH values of the acid-treated samples may have resulted in denaturation of the sarcoplasmic and myofibrillar proteins. The amount of water dispersed among

Table 3. Hunter "a" values of uncooked restructured beef with different treatments.

Treatment	"a" Value	
	$\bar{X}$	S.E.
High collagen unconditioned control	10.59[a]	0.54
High collagen conditioned control	6.65[b]	0.17
Low collagen control	10.73[a]	0.37
High collagen acetic acid treated	4.59[d]	0.18
High collagen citric acid treated	4.33[d]	0.16
High collagen lactic acid treated	5.22[c]	0.20

[abcd] Means in the same column with identical superscripts are not different (P>0.05).

the muscle fibers could affect the reflectance ability of the meat. In a similar study, Whiting and Strange (1988) found lactic acid to have caused some color changes in restructured beef steaks made from muscles that were previously immersed in 0.5M lactic acid for 30 min at 23°C.

CONCLUSION

This research has demonstrated that the incorporation of acetic, citric and lactic acid into restructured beef steaks has the potential of

Table 4. Hunter "a" values of cooked restructured beef with different treatments.

Treatment	"a" value	
	$\bar{X}$	S.E.
High collagen unconditioned control	5.15[a]	0.14
High collagen conditioned control	4.86[a]	0.14
Low collagen control	4.21[b]	0.10
High collagen acetic acid treated	3.07[c]	0.15
High collagen citric acid treated	3.23[c]	0.15
High collagen lactic acid treated	3.29[c]	0.17

[abc] Means in the same column with identical superscripts are not different (P>0.05).

altering properties of the collagen. Further studies to determine the optimum concentration of acid that would cause minimum alterations in the properties of the other proteins are appropriate.

REFERENCES

Bernal, V.M. and Stanley, D.W. 1986. Changes in the melting characteristics of bovine tendon collagen induced by a bacterial collagenase. J. Food Sci. 51:834.Bernal, V.M. and Stanley, D.W. 1987. Stability of bovine muscle connective tissues. J. Food Sci. 52:876.

Cronlund, A.C. and Woychik, J.H. 1987. Solubilization of collagen in restructured beef with collagenases and alpha-amylase. J. Food Sci. 52: 857.

Dransfield, E. and Etherington, D. 1982. Enzymes in the tenderization of meat. In: "Enzymes and Food Processing." G.G. Birch, N. Blankenbrough, and K.J. Parker (Eds.). Elsevier Applied Science Publishers, London. p. 177.

Elkhalifa, E.A. and Marriott, N.G. 1989. Effects of Achromobacter iophagus and splenic pulp on collagen of restructured beef. J. Food Sci. (submitted).

Duncan, D. B. 1955. Multiple range and multiple F tests. Biometry 11:1.

Foegeding, E.A. and Larick, D.K. 1986. Tenderization of beef with bacterial collagenase. Meat Sci. 18:201.

Fogle, 1982. Tenderization of beef: Effect of enzyme, enzyme level, and cooking method. J. Food Sci. 47: 1113.

Fujii, K. and Morota, K. 1982. Isolation of skeletal muscle collagen. Anal. Biochem. 127:449.

Gault, N.F.S. 1984. The influence of acetic acid concentration on the efficiency of marinading as a process for tenderizing beef. Proc. European Meeting of Meat Research Workers No. 30:4:12, p. 184.

Hill, F. 1966. The solubility of intramuscular collagen in meat animals of various ages. J. Food Sci. 31:161.

Hwang, P.T., Addis, P.B., Rosenau, J.R., Nelson, D.A., and Thompson,

D.R. 1977. Use of a rapid muscle protein technique to predict beef muscle yield as a function of time, temperatures, salt and phosphate. J. Food Sci. 42:590.

Robbins, F.M. and Cohen, S.H. 1976. Effects of catheptic enzymes from spleen on the microstructure of bovine semimembranosus muscle. J. Texture Studies 7:137.

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

Strange, E.D. and Whiting, R. C. 1988. Effect of temperature on collagen extractability and Kramer shear force of restructured beef. J. Food Sci. 53:1224.