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I am submitting herewith a thesis written by Cynthia Anne Winstead entitled "Shear Values and Alkali Insoluble Collagen of Beef Biceps Femoris as Affected by Time and Temperature of Heating." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science.

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SHEAR VALUES AND ALKALI INSOLUBLE COLLAGEN OF BEEF BICEPS FEMORIS AS AFFECTED BY TIME AND TEMPERATURE OF HEATING

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Cynthia Anne Winstead

December 1970

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C. A. W.

ABSTRACT

The effect of heating beef cores at 55°, 60°, and 65°C for 30, 60, and 120 minutes was studied. Cores were procured from three pairs of beef biceps femoris muscle from U. S. Choice steers. They were one inch in diameter and were heated in glass tubes in a thermostatically-controlled water bath. Tenderness was evaluated by Warner-Bratzler shear values. The residual alkali insoluble collagen was determined on composite samples of the sheared cores from each heat treatment. Heat treatments were replicated three times, once with each muscle pair.

Cores of muscle heated at 60° and 65°C were more tender than those heated at 55°C, and those heated for 120 minutes were more tender than those heated for 30 minutes ($P<0.05$). The decrease in collagen content of cores heated at 65°C for 120 minutes was greater than the decrease with any other heat treatment. The residual collagen was related to the shear values of the heat-treated cores ($P<0.01$) and accounted for approximately 53 percent of the increase in tenderness.

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CHAPTER I

INTRODUCTION

For many years technologists in meat research have been investigating the underlying factors operating in the tenderness of meat, but results have been inconclusive and much is still to be done. Investigators have become aware that tenderness or toughness of meat is a complex problem that cannot be resolved through the study of any single factor. At least two meat components, muscle fibers and connective tissue, are involved (Ritchey et al., 1963). Connective tissue in muscles is made up of collagen, elastin, reticulin, and the ground substance. Of these constituents, the one present in the largest amount is collagen, which is heat labile (Cover et al., 1962; Ritchey and Cover, 1962; Gould, 1968).

Classically, collagen is protein which may give rise to gelatin on heating in water. Gelatin has been identified as coming from the fibrous material of the connective tissue and tendons of higher vertebrates (Gould, 1968). When meat is cooked or heated, a fraction of the collagen may be converted into gelatin; the amount not converted is believed to be one important factor in the over-all toughness of meat (Ritchey and Cover, 1962; Hamm, 1966). The reactions that

take place during cooking are not well understood in their relationship to tenderness (Tuomy et al., 1963). Hamm (1966) stated that since the myofibrillar proteins are toughened by heat, an increase in tenderness of meat upon cooking must be the result of a change in collagen. Changes in the collagen of connective tissue of beef muscles generally are believed to be initiated at about 64°C. These changes include a softening and transformation of collagen into gelatin thus enhancing tenderness (Bendall, 1964). In a study by Winegarden et al. (1952) sheets of aponeurotic connective tissue were shown to soften on heating in water at 65°C or above for 16 to 64 minutes. Large amounts of connective tissue in a cut of meat could result in an increase in tenderness if conditions permitted the connective tissue to soften. However, with little connective tissue, the effect on the muscle fibers might predominate and the muscle become less tender (Sanderson and Vail, 1963). Cover et al. (1962), in studying the connective tissue component of tenderness in beef, found that a trained panel scored the connective tissue in the longissimus dorsi muscle tender, and that in the biceps femoris tough, when either muscle was heated to 61° or 81°C. However, connective tissue was scored tender in both muscles when heated to 100°C.

Considerable research has attempted to demonstrate a relationship of collagen content of bovine muscle tissue to tenderness of meat (Loyd and Hiner, 1959). Some workers have

indicated a significant negative correlation between alkali insoluble collagen content of raw meat and meat tenderness (Loyd and Hiner, 1959; Cover et al., 1962; Ritchey et al., 1963). However, others have failed to find a clear relationship between collagen solubilization during heating and meat tenderness (McClain et al., 1965; Bayne et al., 1970). Bendall (1964) stated that the toughness of meat cannot be rigidly correlated with collagen content since the cross-bonding parameter is not taken into consideration.

In studies where meat has been cooked according to conventional methods, it is brought to some predetermined end point temperature. If it is a large piece of meat, such as a roast, cores taken from different positions might have had different heat treatments. Since collagen degradation has been shown to be dependent both on time and temperature of heating, the present study investigated the effect of holding meat samples for different time periods at temperatures in the range where collagen denaturation might occur. To achieve this, cores of beef biceps femoris muscle were heated at 55°, 60°, and 65°C for 30, 60, and 120 minutes in glass tubes in a water bath. Heat-treated cores were sheared on a Warner-Bratzler shear apparatus after cooling to room temperature, and then the amount of alkali insoluble collagen in the raw and heated cores was determined. The objectives were to study changes in the amount of alkali insoluble collagen in

beef subjected to the above nine heat treatments and to relate any changes in collagen to tenderness by comparing amount of collagen remaining in heated meat to shear values.

CHAPTER II

REVIEW OF LITERATURE

I. TENDERNESS OF BEEF AS RELATED TO HEAT TREATMENT

Tenderness is one of the most important characteristics of meat. Many studies have been done to determine the factors that are responsible for the tenderness or toughness of beef. It is well established that age, breed, sex of animal, amount of post-mortem aging, the method of cooking, and the particular muscle or muscles used all affect tenderness as perceived by the consumer. In an early study, Satorius and Child (1938) reported that the beef semitendinosus muscle increased in tenderness, as measured by shear force, during cooking until an interior temperature of 67°C was reached; then the muscle became less tender with further heating. They found the longissimus dorsi more tender when heated to 58°C than to 67° or 75°C but the triceps brachii and adductor muscles were less tender when heated to 58°C than when heated to the two higher temperatures.

Bramblett et al. (1959) studied the qualities of beef as affected by cooking at very low temperatures for long periods of time. The adductor, biceps femoris, gracilis,

semimembranosus, and semitendinosus muscles were used. One muscle of each pair was cooked at 63°C for 30 hours and the other at 68°C for 18 hours. Warner-Bratzler shear values and taste panel scores indicated that meat cooked at 63°C was more tender than paired cuts cooked at 68°C. The length of time the meat was held at an internal temperature in the range of 57° to 60°C appeared to be a decisive factor closely related to an increase in tenderness. In another study (Bramblett and Vail, 1964) taste panel scores, chew count, L.E.E. Kramer shear press values, and Warner-Bratzler shear values, indicated that meat cooked at 68°C to an internal temperature of 65°C was more tender than cuts cooked at 93°C to the same end point.

In a study by Tuomy et al. (1963), the effects of cooking temperature over time on tenderness, cuttability, and shear-press values of beef semimembranosus were determined. Cylinders of meat were used for rapid heating and chilling. The cooking temperatures investigated were 60°, 71°, 82°, 88°, 93°, and 99°C over a seven-hour period. The initial effect of heat was a toughening, which increased as the temperature increased. When the meat was held below 82°C, the tenderness of cooked meat depended on temperature, with time having little or no effect. When held at 82°C and above, the meat became tender at a rate and to a degree dependent upon both time and temperature.

Machlik and Draudt (1963) cooked beef semitendinosus cylinders at 1°C intervals between 50° and 90°C for periods up to two or five hours. Minimum shear values were obtained in the range of 60° to 64°C after heating for 30 to 60 minutes.

Fugate (1967) studied the effects of oven and end point temperature on tenderness and other sensory properties of beef rib roasts. Thirty-six roasts were cooked at each oven temperature of 107° and 163°C. End points of 60°, 70°, and 77°C were assigned to paired cuts. Panel scores indicated that roasts cooked at 107°C to each end point temperature and those cooked at 163°C to 60°C were more tender ($P<0.01$) than roasts cooked at 163°C to 70° or 77°C. Roasts cooked at 107°C to 70° and 77°C sheared significantly lower than all others.

Bayne (1968) studied the response of small and large rib and round beef roasts of two degrees of finish to two rates of heat application. Both shear values and tenderness scores indicated that roasts cooked at 93°C, which required about two to three times as long to reach an end point of 70°C, were more tender than those roasted at 149°C.

The physical changes brought about in connective tissue of beef by various heat treatments were studied by Winegarden *et al.* (1952) in an endeavor to relate the effect of heating connective tissue in water to the cooking of meat.

Strips of aponeurotic connective tissue were shown to soften on heating in water at 65°C or above for 16 to 64 minutes. Little softening or other physical change occurred at 60°C or at 65°C with shorter heating periods.

None of the studies just reviewed attempted to explain tenderness on the basis of chemical changes in the proteins of beef. However, investigators have become aware that tenderness or toughness of meat is a complex problem and can only be resolved through the study of many factors.

II. RELATION OF ALKALI INSOLUBLE COLLAGEN TO MEAT TENDERNESS

For many years meat technologists have tried to separate the connective tissue of meat, measure it, and show its relation to tenderness of meat (Loyd and Hiner, 1959). Research workers have learned much about the factors responsible for tenderness.

Husaini et al. (1950) were among the first to attempt to correlate biochemical data with tenderness of beef. Tenderness was determined by taste panel scores. The insoluble protein remaining after extracting with dilute alkali was analyzed for hydroxyproline content and was shown to have a significant negative correlation ($P<0.01$) with tenderness scores.

The relationship between amount of hydroxyproline in alkali insoluble protein and tenderness of three bovine muscles was studied by Loyd and Hiner (1959). Tenderness of the muscles was measured by taste panel ratings and by the Warner-Bratzler shear. Correlations between both types of tenderness measurements and hydroxyproline content were significant ($r = 0.96$ for shears, $P<0.01$; $r = -0.90$ for panel scores, $P<0.05$).

Cover et al. (1962), in studying the connective tissue component of tenderness, found that a trained panel scored the connective tissue in the longissimus dorsi muscle tender, and in the biceps femoris tough, when either muscle was heated to 61° or 80°C. However, connective tissue was scored tender in both muscles when heated to 100°C. Chemical analyses for collagen were made by measuring hydroxyproline in the alkali insoluble residue. Alkali insoluble collagen content was greater in biceps femoris than in longissimus dorsi heated to 61° and 80°C, but there was little difference in collagen in the two muscles heated to 100°C. These findings supported those obtained with the sensory panel.

Ritchey et al. (1963) investigated tenderness of connective tissue in longissimus dorsi and biceps femoris muscles by measuring the collagen content and by panel scores. A significant correlation between the amount of alkali insoluble collagen and meat tenderness was noted. The trends of

panel scores and collagen content indicated increased tenderness at 80°C as compared to 60°C for each of the two muscles.

The collagen content of longissimus dorsi, semimembranosus, and triceps brachii muscles in relation to tenderness was investigated by McClain et al. (1965). Warner-Bratzler shear values were obtained on tissue cooked in deep fat to an internal temperature of 70°C and were used to relate meat tenderness to alkali insoluble collagen content of the raw and cooked meat. Collagen content was similar for raw and cooked meat for triceps brachii and longissimus dorsi muscles, and only slightly different ($P<0.05$) for the semimembranosus muscle. Contrary to previous studies mentioned, results indicated that alkali insoluble collagen content was not related to tenderness for raw or cooked muscles.

The effect of two rates of heat application on the solubility characteristics of bovine intramuscular collagen was investigated by Bayne et al. (1970). Alkali insoluble collagen remaining in paired top round roasts cooked at 93° and 149°C to an end point of 70°C was similar. Correlation coefficients indicated essentially no relationship between amount of alkali insoluble collagen and sensory and mechanical tenderness measurements.

Jackson et al. (1960) reported that there is a continuous spectrum of molecular collagen aggregates with

increasing chronological age marked by an increase in the strength of cross-links and a change in the solubility characteristics of collagen. The solubility of intramuscular collagen in meat animals of various ages was studied by Hill (1966). He found no indication of a systematic increase in total intramuscular collagen with age increase. However, his study provided evidence of an increase with age in the number or strength of the cross-links of intramuscular collagen and furnished a possible explanation as to why total amounts of collagen are inadequate to explain increased toughness associated with increasing chronological age.

III. COLLAGEN MACROMOLECULE CONFORMATION

It is necessary to know the conformation of the molecular chain and the amino acid sequence of collagen to understand collagen activity in biological systems. In recent years the configuration and conformation of collagen have been studied extensively (Ramanathan, 1960).

Classically, collagen fibrils were recognized by their characteristic periodic bands, in which the major bands are separated by distances of approximately 640 to 700 Å, as verified by x-ray diffraction studies. Several models have been proposed to explain this banding, based upon specific modes of aggregation and polymerization of tropocollagen macromolecules to form fibrils (Gould, 1968). The final breakthrough,

which led to the accepted structure of collagen, came not directly from the x-ray pattern, but from stereochemical considerations. These considerations led to a triple helical structure, with the three helices standing side by side and linked to one another by hydrogen bonds nearly perpendicular to the fiber axis (Ramachandran, 1967). Any given collagen from the connective tissue of vertebrates is a unique monomer of molecular weight somewhat less than 300,000. There are generally two kinds of chains in each molecule. These have unique amino acid sequences presumably determined by genetic material in the same manner as other proteins. To produce the functioning unit, the collagen fibril, at least four additional steps following polypeptide synthesis are required. Some of the prolyl and lysyl residues are hydroxylated; carbohydrate is attached; molecules are aggregated in a specific manner into fibrils; and interchain covalent cross-links are produced during and after aggregation. Each of these modifications is presumably necessary for proper function although the reasons for the first two are still matters for speculation. The cross-links bind together the rigid molecular units to produce a structure of low extensibility and high tensile strength (Piez, 1968). The individual helices in the triple helix are not stable since no intrachain hydrogen bonds can be formed between the residues, even by making appreciable distortions (Ramachandran, 1967). However, when three of them are put

together, interhelical hydrogen bonds can be readily formed, so that the triple-helical protofibril is fairly stable.

IV. METHODS OF MEASURING COLLAGEN IN MUSCLE

Because residual collagen is believed to be affected by the method of cooking, considerable attention has been given to the methods of determining collagen. Collagen lacks two amino acids, tryptophan and cysteine, found in most other animal proteins, and has an exceptionally low content of aromatic amino acids. Glycine composes one third, and the imino acids, hydroxyproline and proline, one fifth of the amino acid residues in collagen. Hydroxyproline is found in collagen but in few other proteins. It accounts for approximately 13 percent of the amino acid residues in collagen (Goll et al., 1964). Because of this restricted distribution, hydroxyproline has been widely used as an indicator of both the presence and the metabolism of collagen (Woessner, 1961). In the past 20 years, investigations relating the hydroxyproline content of the alkali insoluble protein of meat to tenderness have been reported (Husaini et al., 1950; Loyd and Hiner, 1959; Ritchey and Cover, 1962). The most commonly employed quantitative assay for this imino acid has been that of Neuman and Logan (1950). Substances other than collagen and elastin are extracted from meat tissue with 0.1 N NaOH at room temperature. After washing the residue with distilled water, it is

hydrolyzed into its constituent amino acids by autoclaving in 6 N HCl. The amount of hydroxyproline in the hydrolysates is then measured. Most of the available methods for the quantitative assay of hydroxyproline depend on one of three colorimetric reactions: reaction with ninhydrin; reaction with isotin; or oxidation of hydroxyproline, which is capable of forming a colored compound (Gould, 1968).

Since histological work was not done in this study, literature pertaining to the effect of heat on histological characteristics of meat has not been discussed.

CHAPTER III

PROCEDURE

I. SOURCE OF MEAT

Three paired biceps femoris muscles from U. S. Choice steer carcasses weighing 600 to 650 pounds were procured through the Missouri Beef Packers in Rockford, Missouri. The muscles were frozen at -29°C in a blast freezer, packaged in freezer paper, and stored at -20°C in a freezer locker until used.

II. PLAN OF STUDY

The entire muscle from right and left sides of one animal was used for each replication. Frozen muscles were sawed across the grain of the muscle fibers into three-inch thick sections from which approximately 80 one-inch cores were removed with the grain of the muscle fibers. Muscle sections were trimmed of external fat and epimysial connective tissue before removal of cores. The muscles were still partially frozen when cores were removed in order to ensure uniformity in diameter. Cores from all sections of both paired muscles were pooled, assigned at random to each treatment, packaged in freezer paper, and frozen until used. The 80 cores from one pair of muscles were used for one replication.

III. HEAT TREATMENTS

On the day prior to starting a test a package containing 20 to 22 cores was removed from the freezer and placed in the refrigerator to defrost. On the day of heating, the cores were trimmed to the same length, approximately two inches. Cores were heated at one of the predetermined temperatures, 55°, 60°, or 65°C, in a constant temperature shaker water bath and six samples removed after 30, 60, and 120 minutes. Cores were heated in uncovered, 50-ml glass Kimax tubes, one inch in diameter. To achieve as uniform heat transfer as possible, a marble was placed on top of each core to help keep the core in contact with the sides of the tube when shrinkage began. The water bath was held at 3°C above the test temperature to maintain the internal temperature of the cores at the test temperature. During the heat treatment, the internal temperature of two cores was measured by copper-constantan thermocouples inserted into the approximate center of the cores and recorded throughout the heating period. Prior to test heating, the cores were brought up to the test temperature in a come-up water bath held at 5°C above the cooking bath temperature, then transferred to the shaker water bath. Heating was stopped immediately after removal from the water bath by submerging the tubes into ice water and holding there for three minutes. Then the cores were removed from the tubes and placed on a tray to cool for one hour at room temperature.

IV. METHODS OF EVALUATION

Physical Evaluation

After cooling for one hour at room temperature, the cooked cores were sheared three times each on a Warner-Bratzler shear. After shear values of the cores were measured and recorded, the sheared cores were frozen and then ground in the frozen state four times through the fine plate of an electric grinder to obtain a homogeneous mixture. Raw cores were ground in the same manner.

Chemical Evaluation

After the meat samples were ground and mixed well, approximately 2-g samples were weighed by difference to the nearest milligram and frozen for lyophilization prior to collagen determination. After lyophilization, the amount of collagen insoluble in 0.1 N NaOH was determined on the raw samples of each pair and on the cooked samples heated at 55°, 60°, and 65°C for 30, 60, and 120 minutes. Analyses were done in duplicate using a modification of the extraction procedure of Ritchey et al. (1963). Collagen was estimated by measuring the hydroxyproline in the acid hydrolysates of the insoluble residue remaining after extraction with sodium hydroxide (Martin and Axelrod, 1953, modification of Neuman and Logan, 1950, procedure).

Extraction and hydrolysis. Lyophilized samples were pulverized with mortar and pestle for 30 seconds and transferred to 50-ml nalgene centrifuge tubes with 35 ml 0.1 N NaOH and stirred vigorously with a glass stirring rod until well mixed. The samples were allowed to stand at room temperature for four hours with occasional stirring. After four hours the tubes were capped securely with parafilm and chilled in the freezer for 30 minutes. The chilled samples were centrifuged at 2100 x gravity for 30 minutes, removed and chilled 15 minutes in the freezer at approximately -20°C, and returned to centrifuge for another 30 minutes. The centrifuge was kept in a refrigerated room. The supernatant was decanted through Gooch crucibles lined with a mat of glass wool, and 35 ml of 0.1 N NaOH was added to the residue left in the tube. The tubes were again capped with parafilm and placed in the shaker water bath at room temperature with the speed control setting at 3.0 for 15 hours. After the second extraction, each supernatant was decanted through the same Gooch crucible used for the first extraction and the residues remaining after two extractions were transferred to the crucibles and washed six times with 25 ml of distilled water at 35°C. The glass wool mat was dried with suction and transferred with tweezers to 50-ml screw-cap test tubes. Ten ml of 6 N HCl were pipetted into the tubes, and the samples were hydrolyzed in an autoclave for eight hours at 15 psi. The hydrolysates were

filtered through folded Whatman 42 filter paper and diluted to 100 ml each. Samples were stored in brown bottles in the freezer until used for hydroxyproline determination.

Hydroxyproline analysis. One-half ml aliquots of the hydrolysates were pipetted in duplicate into 18 x 150 mm Pyrex test tubes and adjusted to 1 ml with distilled water. Aliquots of the operational standard ranging from 5 to 20 µg of hydroxyproline also were pipetted and adjusted to 1 ml with distilled water. Two ml of 1:1 (v:v) mixture of 0.01 M $CuSO_4$ and 10 percent NaOH were added, followed by 1 ml of 6 percent H_2O_2. Immediately after the addition of the peroxide solution, the samples were shaken for five seconds and allowed to stand for 10 minutes. Then the samples were shaken for 15 minutes on a mechanical shaker. One-tenth ml of 0.05 M $FeSO_4$ solution was added to each sample to remove any excess peroxide and shaking was resumed for 10 minutes. Five ml of a 2:3 (v:v) mixture of Ehrlich's reagent and 4 N H_2SO_4 were added, the tubes shaken for 20 seconds, covered with marbles, and held in the shaker water bath at 70°C for 20 minutes for color development. They were removed and cooled in tap water for five minutes. Optical density was read on the Spectronic 20 at 560 mµ. Reagents used in the hydroxyproline analysis are listed in the Appendix.

Nonfat Dry Weight Determination

To have a basis for expressing the percentage of collagen solubilized, fat-free dry weights of approximately 5-g samples were obtained. The samples were weighed by difference to the nearest milligram into Whatman 22 x 80 mm single thickness extraction thimbles. The samples were dried in a vacuum oven for 15.5 hours at 65°C. After cooling in a dessicator the samples were weighed and the percent dry weight determined. The dried samples then were extracted with petroleum ether for six hours on a Goldfisch apparatus. After cooling and evaporating the solvent, the samples were redried, reweighed, and the percent nonfat dry weight was calculated.

Statistical Analyses

Differences in tenderness associated with heat treatments were determined by analysis of variance of shear values. Significance of decreases in collagen in the heat-treated samples also was determined by analysis of variance. Where significance was obtained, Duncan's Multiple Range test was applied. A linear regression was done to determine if there was a correlation between tenderness as measured by the Warner-Bratzler shear and collagen content with the different heat treatments (Steel and Torrie, 1960).

CHAPTER IV

RESULTS AND DISCUSSION

I. EFFECT OF HEAT TREATMENTS ON TENDERNESS

Average shear values for tenderness of the heated cores of meat are presented in Table I. Only the heat-treated cores were sheared. Analysis of variance was done on shear values to determine if there were any differences attributable to animal source, time, temperature, or the interaction of time with temperature (Table II). The analysis indicated differences were associated with animal source ($P<0.01$), as well as with time and temperature of heating ($P<0.05$), but significant differences were not attributable to the interaction of time with temperature. Although differences attributable to time and to temperature were significant, only about 5 percent of the variation in shear values was attributable to the heat treatments applied; about 1.7 percent to time, 1.7 percent to temperature, and approximately 1.6 percent to interaction of time with temperature. Approximately 69 percent of the variation was attributable to variation among cores. This high variation may have been due to inherent differences in various locations of the muscle. Shear value means for temperature without regard to time and for time without regard to temperature are shown in Table III. Cores heated at 60° and 65°C

TABLE I

SHEAR VALUES[a] FOR CORES OF BEEF BICEPS FEMORIS HEATED AT THREE TEMPERATURES FOR THREE TIME PERIODS

Animal number	Heat treatments								
	55°C			60°C			65°C		
	30 min	60 min	120 min	30 min	60 min	120 min	30 min	60 min	120 min
1	22.0	25.1	21.6	22.5	20.8	20.3	23.6	20.4	18.5
2	29.1	22.2	15.0	20.3	21.3	20.5	19.8	21.4	19.5
3	27.1	20.1	21.6	17.3	16.2	15.7	21.3	19.7	14.2
Mean	26.1	22.5	19.4	20.0	19.4	18.8	21.6	20.5	17.4
Standard error	1.6	0.9	0.9	0.7	0.9	0.8	0.9	0.5	0.5

[a]Mean of 18 shears on six cores one inch in diameter.

TABLE II

ANALYSIS OF VARIANCE FOR SHEAR VALUES

Source of variation	d.f.	S.S.	M.S.	F	Percent variation attributable to source
Total	485	23722.30	48.91		
Animal	2	497.71	248.86	5.18 **	2.59
Within Animal	483	23224.58	48.08		
Treatment	24	4712.28	196.34	1.78 *	4.95
Time	6	1462.92	243.82	2.21 *	1.72
Temperature	6	1443.48	240.58	2.18 *	1.68
Time x temperature	12	1805.88	150.49	1.36 NS	1.55
Among cores	135	14887.53	110.28	9.86 **	69.05
Error	324	3624.77	11.19		23.39

*Significant P<0.05

**Significant P<0.01

NSNot Significant

TABLE III

MEAN SHEAR VALUES ASSOCIATED WITH TEMPERATURE AND WITH TIME OF HEATING

Temperature (°C)	Shear value	Time (min)	Shear value
55	22.7	30	22.6[a]
60	19.4[a]	60	20.8[ab]
65	19.8[a]	120	18.6[b]

[ab]Values in the same column with the same superscript do not differ. Other means differ ($P<0.05$). (Duncan's Multiple Range test)

were more tender ($P<0.05$) than those heated at 55°C. Cores heated for 120 minutes were more tender than those heated for 30 minutes, but those heated for 30 and 60 minutes were similar as were those heated for 60 and 120 minutes. The effects of time and temperature on shear values are illustrated in Figure 1. A trend toward increased tenderness with increased time was observed at all three temperatures with the greatest effect at 55°C and the least at 60°C.

The data in this study are not in agreement with those of Tuomy et al. (1963) who cooked cores of beef at several temperatures between 60° and 99°C over a seven-hour period. When the meat was held below 82°C the tenderness depended on temperature with time having little or no effect. When held at 82°C and above, tenderness was dependent upon both time and temperature. The present study agrees to some extent with that of Machlik and Draudt (1963) who cooked beef cylinders at temperatures between 50° and 90°C for periods up to two or five hours and found minimum shear values in the range of 60° to 64°C after heating for 30 to 60 minutes.

II. EFFECT OF HEAT TREATMENTS ON COLLAGEN CONTENT OF BICEPS FEMORIS MUSCLE

In this study the effect of time and temperature of heating on the amount of residual (alkali insoluble) collagen of beef biceps femoris also was investigated. The amount of

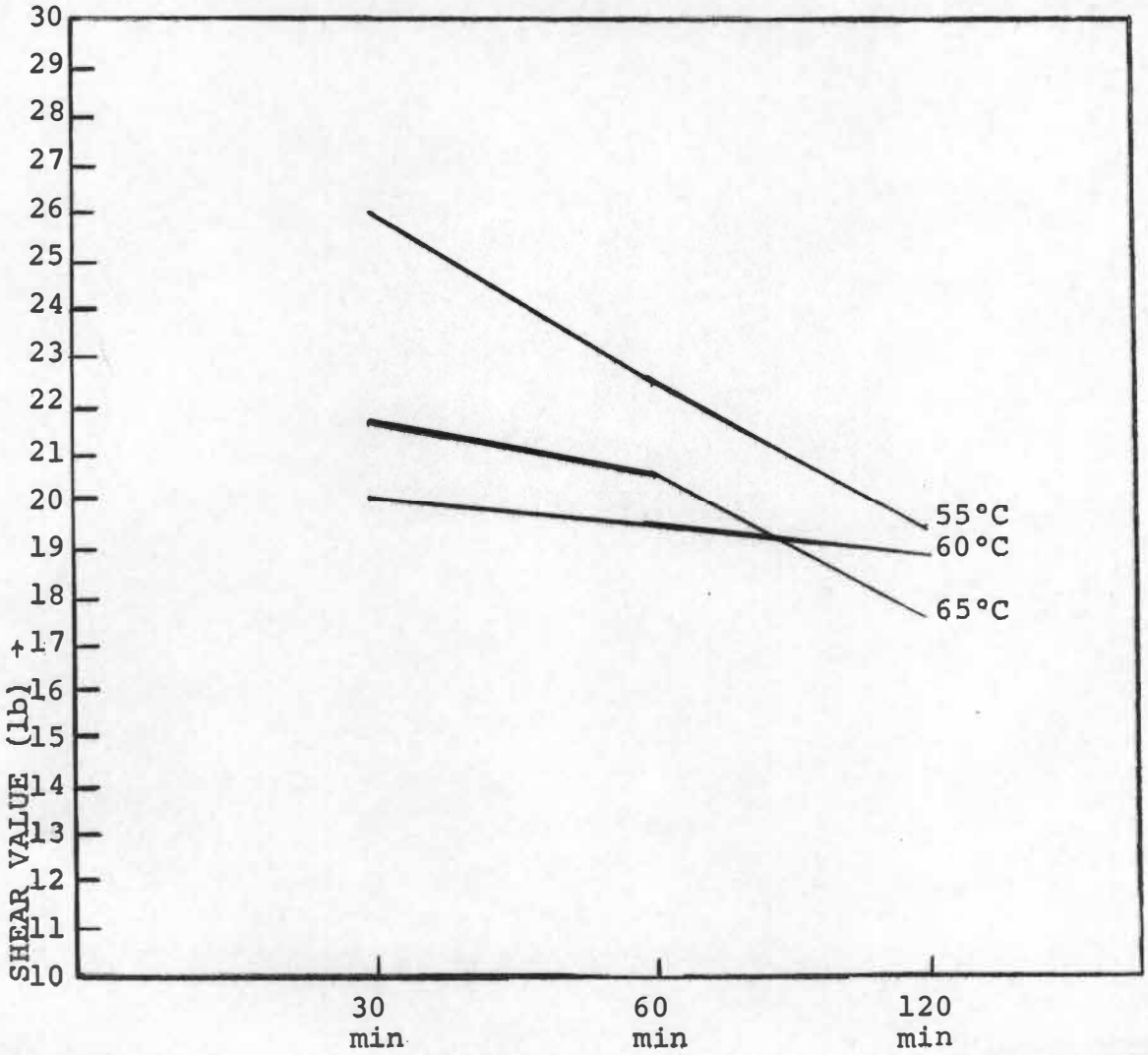


Figure 1. Effect of time and temperature of heating on shear values of cores of beef biceps femoris.

collagen on a nonfat dry weight basis in the raw and heated muscle is presented in Table IV. An analysis of variance was done on the decreases in collagen content from raw to heated tissue. These decreases are presented in the Appendix in Table VIII. Mean squares from the analysis of variance are presented in Table V. Significant differences were associated with time, temperature, and the interaction of time with temperature of heating ($P<0.001$). As indicated in Table V approximately 95 percent of the variance in collagen decreases was attributable to the heat treatments applied, of which about 20 percent was attributable to time of heating, about 42.5 percent to temperature of heating, and approximately 32.5 percent to the interaction of time with temperature. In Table VI mean values for collagen decrease for temperature without regard to time and for time without regard to temperature are shown. The increased amounts of collagen solubilized with each increase in temperature were all very highly significant. There was no difference in the amounts of collagen solubilized with 30 and 60 minutes of heating, but the decrease in collagen with 120 minutes of heating was greater than with either 30 or 60 minutes ($P<0.001$). Ranked means for collagen decreases for the nine heat treatments are shown in Table VII. The decrease in collagen in muscle tissue heated at 65°C for 120 minutes was found to be greater than for all other heat treatments ($P<0.001$). The greatest difference in collagen

TABLE IV

ALKALI INSOLUBLE COLLAGEN CONTENT[a] OF RAW AND HEATED BEEF BICEPS FEMORIS MUSCLE

Animal number	Collagen (mg/g, NFD basis)									
		55°C			60°C			65°C		
	Raw	30 min	60 min	120 min	30 min	60 min	120 min	30 min	60 min	120 min
1	36.4	33.6	42.0	32.6	31.5	28.8	29.5	32.2	28.7	26.2
2	33.5	33.4	29.4	22.0	28.4	29.9	26.3	25.4	28.4	22.8
3	31.4	29.9	27.7	30.6	27.0	24.5	22.8	23.8	22.0	12.7
Mean	33.9	32.3	33.0	28.4	29.0	27.7	26.2	27.1	26.4	20.6
Standard error	0.6	0.8	2.9	2.1	0.9	1.1	1.2	1.6	1.4	2.6

[a]Mean of six values for raw, duplicate values for heat-treated

TABLE V

ANALYSIS OF VARIANCE FOR COLLAGEN DIFFERENCES

Source of variation	d.f.	S.S.	M.S.	F	Percent variation attributable to source
Total	53	1052.60	19.86		
Animal	2	43.62	21.81	1.10 NS	1.03
Within Animal	51	1008.98	19.78		
Treatment	24	996.78	41.53	91.95 ***	94.81
Time	6	235.16	39.19	86.77 ***	19.79
Temperature	6	501.50	83.58	185.04 ***	42.48
Time x temperature	12	260.13	21.68	47.99 ***	32.54
Error	27	12.20	0.45		4.13

NS Not significant

*** P<0.001

TABLE VI

MEAN COLLAGEN DECREASES[a] ASSOCIATED WITH TEMPERATURE AND WITH TIME OF HEATING

Temperature (°C)	Decrease	Time (min)	Decrease
55	2.6[b]	30	4.4[b]
60	6.2[c]	60	4.8[b]
65	9.2[d]	120	8.8

[a]Decrease from values for raw sample (mg/g, NFD basis)

[b]Values in the same column with the same superscript do not differ. Other means differ ($P<0.001$). (Duncan's Multiple Range test)

TABLE VII

RANKED MEANS FOR COLLAGEN DECREASES[a] IN BEEF BICEPS FEMORIS CORES GIVEN NINE HEAT TREATMENTS

Heat treatments (°C/min)	55/60	55/30	60/30	55/120	60/60	65/30	65/60	60/120	65/120
Mean values	0.9[b]	1.6[b]	4.9[c]	5.5[cd]	6.2[cde]	6.8[de]	7.5[e]	7.7[e]	13.3
Standard error	2.0	0.5	0.4	2.1	0.8	0.8	0.7	0.4	1.7

[a]Decrease from corresponding value for raw sample (mg/g, NFD basis)

[bcde]Mean values with the same superscript do not differ. Other means differ ($P<0.001$).

decrease was between cores heated at 55°C for 30 and 60 minutes and those heated at 65°C for 120 minutes ($P<0.001$). At each temperature, 55°, 60°, and 65°C, collagen decreases at 30 and 60 minutes did not differ, although the decrease at 55°C was less than at either 60° or 65°C. The decrease in collagen of cores heated for 120 minutes was greater ($P<0.001$) than that of cores heated for 30 minutes at any of the three temperatures. The decrease in collagen of cores heated at 55°C for 120 minutes, 60°C for 30, 60, and 120 minutes, and 65°C for 30 and 60 minutes was greater than decreases at 55°C for 30 and 60 minutes ($P<0.001$). Figure 2 shows the effect of time and temperature of heating on decreases in collagen content. The importance of temperature to reduction of collagen is illustrated.

III. RELATION BETWEEN TENDERNESS MEASUREMENTS AND COLLAGEN CONTENT OF HEAT-TREATED MUSCLE

A linear regression was done to determine if there was a correlation between tenderness values as measured by the Warner-Bratzler shear and collagen content of cores with the nine heat treatments. A significant relation ($r = 0.73$; $P<0.01$) was found between shear values and collagen content of the heat-treated muscle. An r^2 of 0.5335 was obtained, indicating that 53.35 percent of the variation in shear values could be accounted for by variation in collagen content.

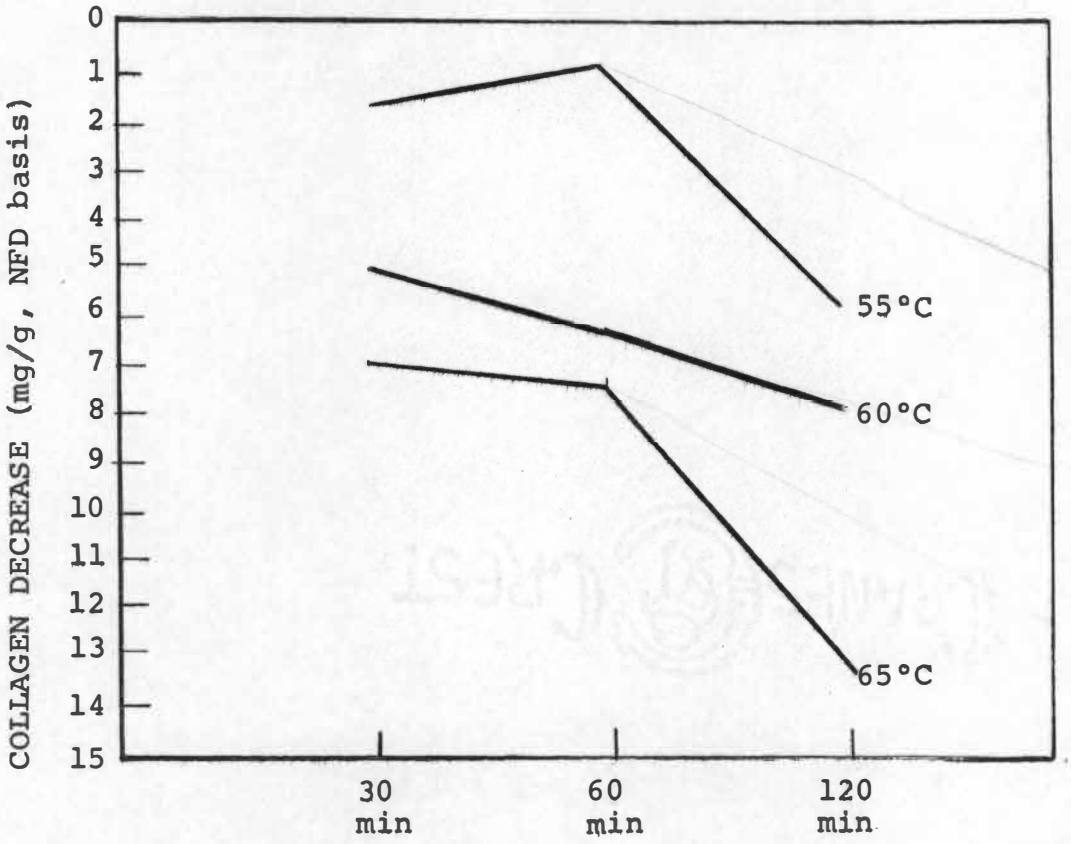


Figure 2. Effect of time and temperature of heating on collagen decreases in beef biceps femoris.

These findings are in agreement with Loyd and Hiner (1959) who found a significant correlation ($P<0.01$) between Warner-Bratzler shear values and hydroxyproline content of alkali insoluble protein of three bovine muscles. Present findings are also in agreement with Cover et al. (1962), who found the connective tissue in the longissimus dorsi muscle tender, and that in the biceps femoris tough, when either muscle was heated to 61° or 81°C. Connective tissue was tender in both muscles when heated to 100°C. Alkali insoluble collagen content was greater in biceps femoris than in longissimus dorsi heated to 61° and 81°C, but there was little difference in collagen in the two muscles heated to 100°C. However, present data are not in agreement with a study by McClain et al. (1965), in which Warner-Bratzler shear values were used in relating meat tenderness to alkali insoluble collagen content of beef. They indicated that alkali insoluble collagen content was not related to tenderness of raw or cooked muscles. Likewise, the present study does not agree with that of Bayne et al. (1970) in which correlation coefficients indicated essentially no relationship between amount of alkali insoluble collagen and either sensory or mechanical tenderness measurements in top round roasts cooked at 93° and 149°C to an end point of 70°C.

IV. DISCUSSION

The data obtained in this study indicated that both time and temperature of heating affected the tenderness of the meat. Tenderness as measured by Warner-Bratzler shear values increased as time of heating increased from 30 to 120 minutes and also increased as temperature increased from 55° to 60° or 65°C. Collagen content decreased as time and temperature of heating increased from 55° to 65°C and from 30 to 120 minutes with temperature of heating exerting a greater effect than the length of the heating period. A correlation ($r = 0.73$; $P<0.01$) between collagen content and shear values was obtained. Since only 53 percent of the variance in shear values was attributable to the decrease in collagen, other factors cannot be ruled out as contributors to the tenderizing effects of the heat treatments studied. Further studies using the technique of tube heating seem feasible. Since there was so much difference in shear values among individual cores, it is recommended that a more uniform muscle be used in further work.

CHAPTER V

SUMMARY

I. SCOPE OF THE STUDY

Three pairs of beef biceps femoris muscles from U. S. Choice steer carcasses were used in the study. Cores one inch in diameter from each pair of muscles were heated at 55°, 60°, and 65°C for 30, 60, and 120 minutes in glass tubes in a constant temperature water bath. The amount of alkali insoluble collagen in the raw and heated muscle tissue was determined by analyzing alkali insoluble residue for hydroxyproline. Collagen content of the heated muscle tissue was compared to tenderness as measured by a Warner-Bratzler shear.

II. PRINCIPAL FINDINGS

Biceps femoris cores heated at 55°C were less tender than those heated at 60° or 65°C. Cores heated for 30 minutes were less tender than those heated for 120 minutes. The greatest decrease in collagen content of heated tissue was in muscle given the most severe heat treatment, 65°C for 120 minutes. The least decrease in collagen was found in muscle heated at 55°C for 30 and 60 minutes. A correlation between collagen content of heated muscle tissue and shear values was obtained ($r = 0.73$; $P<0.01$).

III. IMPLICATIONS

Heating biceps femoris cores for 120 minutes increased tenderness more than heating for 30 minutes, and cores heated at 60° or 65°C were more tender than those heated at 55°C. Collagen content of the cores decreased as time and temperature of heating increased with temperature of heating exerting the greater effect. The data imply that some insight as to the role of collagen in increased tenderness upon heating of bovine muscle may be obtained by studying the relationship of shear values to collagen solubility in dilute alkali. During this study considerable variation was observed within a muscle, and shear values varied widely among cores of the same muscle given the same heat treatment. On the basis of the data obtained, it appears that studies of collagen solubility in a more uniform muscle would be justified.

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APPENDIX

REAGENTS FOR HYDROXYPROLINE ANALYSIS

1. Standard Hydroxyproline Stock Solution: 250 mg of Hydroxy-L-Proline was dissolved in distilled water and diluted to 1000 ml. Portions of 100 ml were stored in brown bottles in a freezer.

2. Operational Standard Solution: 10 ml of the stock solution and 10 ml of 6 N HCl were diluted to 100 ml with distilled water. Made fresh for each determination.

3. Copper Sulfate Solution (0.01 M): 2.5 g of $CuSO_4 \cdot 5 H_2O$ were dissolved in sufficient water to make 1000 ml.

4. Sodium Hydroxide Solution (10 percent): 100 g of NaOH (97.3 percent pure) were dissolved in sufficient water to make 1000 ml.

5. Hydrogen Peroxide Solution (6 percent): 20 ml of 30 percent H_2O_2 were diluted to 100 ml with distilled water. Made fresh for each determination.

6. Ferrous Sulfate Solution (0.05 M): 1.4 g of $FeSO_4 \cdot 7 H_2O$ and 0.5 ml concentrated H_2SO_4 were dissolved in distilled water and diluted to 100 ml. Made fresh for each determination.

7. Ehrlich's Reagent (5 percent): 25 g of para-dimethylaminobenzaldehyde were dissolved in sufficient 1-propanol to make 500 ml.

8. Sulfuric Acid (4 N): 110 ml of 97 percent H_2SO_4 (sp. gr. 1.84) were diluted to 1000 ml with distilled water.

TABLE VIII

DECREASES IN COLLAGEN CONTENT[a] OF BEEF BICEPS FEMORIS MUSCLE RECEIVING NINE HEAT TREATMENTS

Animal number	Heat treatments								
	55°C			60°C			65°C		
	30 min	60 min	120 min	30 min	60 min	120 min	30 min	60 min	120 min
1	2.9	5.6[b]	3.8	5.0	7.7	6.9	4.3	7.7	10.2
2	0.4	4.5	11.8	5.4	3.9	7.5	8.4	5.4	11.0
3	1.4	3.7	0.8	4.4	6.9	8.6	7.6	9.4	18.7
Mean	1.6	0.9	5.5	4.9	6.2	7.7	6.8	7.5	13.3
Standard error	0.5	2.0	2.1	0.4	0.8	0.4	0.8	0.7	1.7

[a]Decrease from corresponding values for raw sample (mg/g, NFD basis)

[b]Increase

VITA

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