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Changes in Tenderness and Collagen of Beef Semitendinosus Muscle Heated at Two Rates

Marjorie Porter Penfield
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To the Graduate Council:

I am submitting herewith a dissertation written by Marjorie Porter Penfield entitled "Changes in Tenderness and Collagen of Beef Semitendinosus Muscle Heated at Two Rates." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Food Science and Technology.

Bernadine Meyer, Major Professor

We have read this dissertation and recommend its acceptance:

Gracyce E. Goertz, Ada Marie Campbell, J. O. Mundt, William Backus

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

November 16, 1973

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Accepted for the Council:

Vice Chancellor for
Graduate Studies and Research

132

CHANGES IN TENDERNESS AND COLLAGEN OF BEEF SEMITENDINOSUS MUSCLE HEATED AT TWO RATES

A Dissertation

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

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by

Marjorie Porter Penfield

December 1973

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ABSTRACT

Tenderness, an important quality attribute of meat, is affected by many factors, including rate of heating. The purpose of this investigation was to study the changes occurring in beef semitendinosus muscle and intramuscular connective tissue heated at rates comparable to oven roasting at 93 and 149°C. The sequence of changes occurring during heating was followed by evaluating samples heated to four end points, 40, 50, 60 and 70°C. Cores of meat and isolated connective tissue samples in buffer were heated in a water bath "programmed" to produce the desired rate of heating.

As internal temperature increased Warner Bratzler shear values of cores decreased ($P < 0.001$). Slow heating produced more ($P < 0.05$) tender cores than faster heating. Shear values were negatively related ($P < 0.05$) to percent connective tissue solubilized during heating. More ($P < 0.01$) connective tissue was solubilized in slowly heated cores and solubilization increased ($P < 0.001$) with internal temperature.

Enzyme activity was exhibited in cores heated to all end points but decreased ($P < 0.01$) from 60 to 70°C. Small amounts of activity were found in the drip lost during heating. Activity in the drip decreased ($P < 0.05$) with heating, more slowly ($P < 0.05$) at the slow rate than at the fast rate of heating. It is postulated that enzyme activity could affect the difference in tenderness between the slow and fast heated cores.

Isolated connective tissue heated in buffer was solubilized to a greater ($P < 0.001$) extent when heated at the slow rate than at the fast rate. Solubilization increased ($P < 0.001$) as end point temperature increased. Peptides in samples heated to 70°C were estimated to be longer ($P < 0.05$) than those heated to lower temperatures. Solubility of the heated connective tissue in guanidine hydrochloride decreased ($P < 0.001$) with heating and to a greater ($P < 0.05$) extent in the slowly heated samples.

From the results of this study it would appear that solubilization of connective tissue is not the only factor affecting the increased tenderness of slowly heated meat. General proteolytic activity may play a role in this increased tenderness. Further investigation of the residual connective tissue and the effects of the two heating rates on myofibrillar proteins is needed to explain the differing effects of slow and fast rates of heat penetration.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

TABLE PAGE

TABLE PAGE

CHAPTER I

INTRODUCTION

The effect of heating on the tenderness of meat is an extremely complex problem. Although tenderness related changes occurring in meat during heating have been studied extensively many questions remain unanswered. Several including Paul (1963), Hamm (1966) and Draudt (1972) have theorized that heat related changes in meat tenderness result from two opposing effects. Changes in connective tissue have a tenderizing effect while hardening of the myofibrillar proteins has a toughening effect.

Roasting of beef at very low oven temperatures (66-121°C) for long periods of time produced more tender meat than roasting at a higher temperature (149-163°C) for a shorter period of time (Bramblett et al., 1959; Bramblett and Vail, 1964; Bayne et al., 1969). The slower rate of heat penetration resulting in a prolonged period of time in the 57-60°C range may promote greater degradation or softening of connective tissue without extensive hardening of muscle fibers (Paul, 1963).

In studies focusing on changes in connective tissue, alkali insoluble collagen decreased during heating (Ritchey and Cover, 1962; Ritchey et al., 1963; McClain et al., 1965a; Bayne et al., 1971). However, Bayne et al. (1971) did not find that the amount of residual collagen differed with respect to heating rate.

Tenderness of meat has been related to the cross linkages of collagen in a number of studies. These studies have been related to changes in chronological age (Hill, 1966; Herring et al., 1967) and to changes in tenderness as a result of varying methods of suspension during post mortem aging (Kruggel and Field, 1971). Little work on cross linkage in relation to changes occurring during heating was reported in the literature.

Increased tenderness of meat cooked at very low temperatures for long periods of time may be attributable partially to the action of enzymes. Laakkonen et al. (1970b) reported the presence of proteolytic and collagenase-like enzymatic activity in meat heated at slow rates. Further work is needed to confirm these results in order to better define the role, if there is one, of enzymes in the tenderization of meat during long, slow heating.

The present investigation focused on changes in the connective tissue component of semitendinosus cores heated in a water bath at rates comparable to oven roasting at 93°C (200°F) and 149°C (300°F) in relation to changes in tenderness as measured by Warner Bratzler shear. To evaluate these changes progressively, samples were heated to four end point temperatures, 40, 50, 60 and 70°C. Changes in connective tissue heated in the intact muscle tissue and in connective tissue isolated from muscle tissue prior to heating were both evaluated. Solubilization of connective tissue in the intact tissue was studied and more specific changes in the collagen molecule were studied in the isolated connective tissue. The occurrence of general

proteolytic activity in the muscle and in the drip lost during heating also was investigated.

CHAPTER II

REVIEW OF LITERATURE

Tenderness has been repeatedly described as the most important quality attribute of meat to the consumer, overshadowing color and flavor (Bailey, 1972). Factors affecting tenderness are many. Paul (1972) discussed the following as factors that influence the tenderness of meat: genetic factors including species, breed and sire; animal influences including feed, exercise and stress; slaughter and post mortem treatment; muscle structure, composition and function; and processing and cooking methods. These factors have been studied in relation to the two components of muscle that are responsible for the tenderness or lack of tenderness of a piece of meat. The effect of heating on tenderness and the effect of heating on the connective tissue component of muscle have been the subjects of many investigations. Limited work on the role of enzymes in the tenderization of meat during heating has been reported.

I. THE EFFECT OF HEATING ON THE TENDERNESS OF BEEF

In an early study, Sartorius and Child (1938) reported that semitendinosus muscle heated at 150°C increased in tenderness when heated to 58 and 67°C. A decrease in tenderness was noted at 75°C. Muscle fiber diameters decreased up to 67°C and remained constant from 67 to 75°C. In relating these changes in tenderness to alterations in muscle fibers and connective tissue, the investigators suggested that

during heating to 58 and 67°C, hydrolysis of collagen was evidently more effective than increased density due to muscle fiber coagulation as a determinant of tenderness. The reverse was true from 67 to 75°C. They postulated that it might be expected that very tender cuts containing little collagen would decrease in tenderness during heating whereas less tender cuts would become more tender.

Roasting of beef at very low oven temperatures (66-121°C) for long periods of time has been shown to produce more tender meat than higher temperature (149-163°C), shorter time methods of oven roasting in a number of studies. Time rather than oven temperature was suggested by Cover (1943) as the important factor in tenderizing during heating. When the rate of heat penetration was low enough to require 30 hours or more to reach the well done stage, a tender product was always produced. Roasts were not always scored as tender if less time was required. Roasts heated to well done in an 80°C oven were scored more tender subjectively and objectively than the pair mates heated in a 125°C oven.

U. S. Standard grade beef round roasts cooked at 63°C for 30 hours were more tender than the pair mates heated in a 68°C oven for 18 hours (Bramblett et al., 1959). The decisive factors appeared to be the length of time that the meat was held in the 57 to 60°C range. Shear values decreased ($P < 0.01$) as the length of time in this temperature range increased. Changes in both the muscle fiber and connective tissue components resulting in more tender meat seemed to be occurring in this temperature range.

In a later study, Bramblett and Vail (1964) found that muscles from rounds of U. S. Good grade carcasses heated to an internal temperature of 65°C in a 69°C oven were more tender than the pair mates heated in a 93°C oven to the same end point. Heating times were two to four times longer in the 69°C oven.

Bayne et al. (1969) studied the effect of two rates of heating on paired large and small rib and top round roasts. Oven temperatures of 93°C and 149°C were used to heat the roasts to end points of 67°C and 70°C respectively. Roasts heated at the slower rate were more ($P < 0.001$) tender than those heated at 149°C. Both shear values and panel scores for tenderness showed this difference. As in several other studies, the authors postulated that the slower rate of heat penetration at 93°C could have promoted increased degradation of collagen resulting in a more tender product.

In a study of the effect of low-temperature, long-time heating on bovine muscles, Laakkonen et al. (1970a) noted an increase in tenderness with heating. Slices of semitendinosus muscle were heated in a water bath increasing in temperature at a rate of 0.1°C per minute to 60°C and held for a total cooking time of 10 hours. The major increase in tenderness occurred between the fourth and sixth hours of heating. The temperature of the meat increased from 50 to 60°C during that period of time.

In order to define the effect of various time-temperature treatments on the shear values of semitendinosus muscle, Machlik and Draudt (1963) chose to heat small cores of meat in test tubes in a water bath.

Cores were heated for varying lengths of time at temperatures ranging from 50 to 90°C. A decrease in shear became apparent in the 55-56°C range and was attributed to the collagen shrinkage reaction. Time required for completion of this reaction varied with temperature. In the 57-59°C range one hour was required whereas the reaction was essentially complete in 15 minutes or less at 60-65°C. Shear values increased from 66 to 80°C and began to decrease again in the 80-90°C range.

The results of this study and other work from his laboratory led Draudt (1972) to conclude that the shear values of meat cooked at various temperatures reflect changes occurring in muscle fibers and connective tissue during the heating process. He proposed that the following factors contribute to changes in shears at various temperatures. At 40°C the mechanical properties of meat have not been significantly affected by heat; therefore, shears at this point are indicative of initial tenderness. At 50°C denaturation of most of the contractile proteins has occurred. Collagen shrinkage or solubilization and hardening of the myofibrillar proteins have not yet occurred. Therefore shear values are at a maximum point prior to collagen shrinkage. Shear values of meat heated at 60°C reflect the effect of the collagen shrinkage reaction without any appreciable myofibrillar hardening. At 74°C the magnitude of hardening and limited collagen solubilization can be seen. Solubilization of collagen may be reflected at 94°C.

Hamm (1966) also concluded that changes in the myofibrillar and connective tissue components can be broken into steps according to temperatures of heating. He used ranges rather than individual points in his description of the changes that occur. From 20-30°C changes in these two components are not evident. Myofibrillar changes in the 30-50°C range include an unfolding of peptide chains. A tighter network of protein structure results from the formation of unstable cross linkages. These changes affect the rigidity of the tissue. Stable cross links form from 50 to 55°C. At 65°C coagulation of most of the myofibrillar and globular muscle proteins has occurred. At temperatures around 63°C collagen shrinkage occurs. Hamm stated that at higher temperatures an increase in tenderness attributable to collagen transformation to gelatin may occur.

II. THE STRUCTURE AND PROPERTIES OF COLLAGEN IN RELATION TO TENDERNESS

Collagen, the major component of white connective tissue, is present in varying quantities in animal tissue. Its composition may vary slightly from species to species but certain basic characteristics can be enumerated. Glycine is present as every third residue in a polypeptide chain of the collagen molecule, except in a small atypical, non-helical portion of the chain. Collagen contains two amino acids of unique character. Hydroxyproline and hydroxylysine represent approximately 25 residue percent. Triplets of the polypeptide chain can be represented by the scheme, -GLY-X-Y-. Hydroxyproline is found

only in the Y position whereas proline may be found in the X or the Y position (Veis, 1970).

The collagen molecule is composed of three helical polypeptide chains. The chains are wound around each other in a type of coiled coil. A regular array of hydrogen bonds within the molecule serves a stabilizing function. Stereochemical restrictions due to the high content of pyrrolidine rings of proline and hydroxyproline also contribute to the stability of the molecule. The exact molecular weight of collagen is unknown but is somewhat less than 300,000 (Piez, 1966; Veis, 1970).

Individual collagen molecules are linked together by a system of intermolecular cross linkages. Harding (1965) explained that the insolubility of all but a small portion of mature collagen fibers in any aqueous or organic solvent that does not attack collagen chemically is an indication of a highly cross linked system. Small portions of collagen can be extracted from collagenous materials with dilute acid buffers, alkali buffers and salt solutions. These extracts contain individual trimers, dimers and monomers. Such soluble components can have no covalent intermolecular cross links. Mature collagen fibers are insoluble in dilute acid and salt solutions (Schubert and Hamerman, 1968).

Bornstein et al. (1966) noted that disulfide bonds are not possible in collagen since cystine is present in insignificant amounts. The nature of cross linking has been difficult to study. Consequently, exact mechanisms have not been described.

Of particular interest to meat scientists are studies regarding the occurrence of aldehyde derived cross linkages in collagen. Early evidence for this type of linkage was noted in studies regarding the disorder lathyrism (Page and Benditt, 1967; Levene, 1962). The condition is characterized by a dramatic increase in the amount of soluble collagen present in tissues. Lathyrism has been induced chemically in experimental animals with beta aminopropionitrile fumarate which inhibits aldehyde formation from lysine in a peptide linkage. Piez (1968) noted that both intra- and intermolecular cross linkages are inhibited by lathyrogenic compounds and concluded that aldehyde formation is involved in both cross linking processes.

Bornstein et al. (1966) pointed out that evidence from ^{14}C-lysine studies suggested that a cross link is formed in collagen via an aldol type condensation of two lysine derived aldehydes in separate protein chains to produce a new aldehyde. The new aldehyde in vivo or in the process of extraction became unsaturated to form an α,β-unsaturated aldehyde. The exact chemistry of the formation of the bond was not determined. The investigators suggested that the aldehyde linkage may occur within the collagen molecule involving lysine residues in the atypical end of the molecule. An intermolecular link may involve a condensation of a lysine derived aldehyde in the atypical portion of one molecule with the ϵ-amino group of lysine in the helical portion of another molecule.

Kruggel et al. (1970) used several methods to study the molecular structure of epimysial acid soluble collagen (ASC) from meats of varying

tenderness. ASC is a soluble form of collagen that still contains appreciable amounts of cross links. ASC with a lesser degree of cross links as measured by sucrose-density gradient ultracentrifugation was extracted from epimysial connective tissue of more tender meat. Intrinsic viscosity studies confirmed this finding. Lysine occurred in greater amounts in ASC from less tender meat suggesting a potential for more frequent aldehyde linkages. Chemical estimation of the aldehyde type cross linkages with 2,4-dinitrophenylhydrazine showed that epimysial ASC from less tender meat contained more aldehyde than that of more tender meat. The workers emphasized that the collagen studied was epimysial rather than intramuscular in origin. The intramuscular collagen would have a more direct influence on tenderness. Furthermore, all collagen in a tissue might be related and therefore exhibit the same degree of cross linking.

Kruggel and Field (1971) reported a decrease in the aldehyde content of guanidine hydrochloride soluble intramuscular collagen (GSIC) extracted from muscles that were stretched during aging. Guanidine hydrochloride is a denaturing agent capable of extracting high molecular weight aggregates of collagen containing inter- and intramolecular cross links. Tenderness was greater in muscles that were stretched during aging than in those aged normally.

In a comparable study, Pfeiffer et al. (1972) reported similar but nonstatistically significant changes. The α or single chain component of GSIC increased with stretching as well as with aging for 21 days. As the amount of the α component present increased, shear

values of steaks prepared from adjacent areas of the muscle decreased. Shear values decreased as the amount of γ component or trimers decreased. This constituted evidence that molecular configuration of intramuscular collagen is associated with tenderness. Stretching had no effect on the amount of labile collagen released from GSIC samples during heating in Ringer's solution. Similarities in the composition of the soluble collagen fractions studied indicated that if differences in covalent cross linking occur they must be within the insoluble collagen fractions.

Most studies regarding cross linking of collagen in relation to tenderness or factors affecting it have taken a less direct approach. Heat labile collagen, the fraction solubilized during heating in Ringer's solution or in water, is an indirect measure of cross linking thought to be related to tenderness. As the extent of cross linking increases the proportion of heat labile collagen decreases.

Chronological age also is a factor in the tenderness of meat. Changes in collagen with age may be responsible for the decrease in tenderness. Herring et al. (1967) reported that collagen solubility decreased with advancing chronological age. Collagen solubility was greater in the longissimus than in a less tender muscle, the semimembranosus.

Hill (1966) also reported that while there is no increase in the total collagen content with increasing chronological age, solubility of the collagen decreases indicating the formation of more frequent and stronger cross bonds. Therefore, the degree of solubility of

collagen as well as the total content should be considered in studies of the cause of lack of tenderness in meat.

Similar findings were noted by Goll et al. (1964a). Collagen residues from animals in four groups representing advancing maturity were heated at varying temperatures in a buffered medium of pH 7. As age increased, the amount of soluble materials released decreased. In addition, advancing age resulted in an increase in the thermal shrinkage temperature. For the reported work, the thermal shrink temperature was that temperature at which a sudden release of soluble hydroxyproline containing materials occurred.

Collagen is not a simple component of the muscle. It may consist of a number of forms of the molecule ranging from the soluble forms to the very insoluble forms. Several of these fractions of intramuscular collagen have been studied in relation to tenderness. McClain et al. (1965b) found no significant differences among tender and less tender muscles with respect to acid and salt soluble collagen content.

Alkali insoluble collagen in raw meat has been investigated in attempts to define the role of collagen in tenderness of heated meat. A significant negative correlation between alkali insoluble protein and tenderness at 14 days post mortem was noted by Husaini et al. (1950). A very high coefficient of correlation of -0.87 was found indicating that the alkali insoluble protein fraction was associated with tenderness in meat. Short loins from twenty animals representing a variety of market grades were included in the study.

Comparable findings were described by Loyd and Hiner (1959). Longissimus, semitendinosus and psoas major muscles were evaluated. Highly significant correlations between hydroxyproline content of alkali insoluble protein and mechanical shear as well as taste panel ratings were shown.

McClain et al. (1965a) did not find a significant difference in alkali insoluble collagen content of raw longissimus and triceps brachii from tender and less tender carcasses. Slight differences were found in the collagen content of semimembranosus muscles from these same carcasses. Significant differences were found between muscles within tenderness groups. Alkali insoluble collagen content was not related to shear values in this investigation.

Conflicting results are reported above and others were found in the literature. Therefore, it is impossible to make any definite statement regarding the role of alkali insoluble collagen content of raw meat in the tenderness of heated meat.

III. THE EFFECT OF HEAT ON COLLAGEN IN RELATION TO TENDERNESS OF BEEF

The effect of heat on collagen has been studied extensively in attempts to explain the mechanism or mechanisms responsible for changes in tenderness of meat with heating. Two approaches have been taken. A few have involved changes in isolated connective tissue subjected to heating while others have involved changes in the connective tissue in intact muscle tissue.

Winegarden et al. (1952) heated strips of tissue composed mainly of connective tissue in distilled water for varying periods of time at a number of different temperatures. At 60°C little or no softening occurred. Softening was evaluated with the Warner Bratzler shear. The critical temperature for softening was 65°C since the change occurred in a short period of time at this temperature. At 80°C extensive changes occurred in one minute. If the connective tissue in intact muscle behaved in a like manner little change in tenderness would occur in steaks and roasts heated to rare (55°C) and medium (65°C). However, it was noted that longer times of 16 and 64 minutes at 65°C resulted in an increased degree of softening.

Longissimus dorsi contained less alkali insoluble, autoclave soluble collagen nitrogen than biceps femoris in a study by Ritchey et al. (1963). This relationship was shown in raw steaks and in steaks cooked to 61 and 80°C. Collagen nitrogen decreased during heating and with increasing internal temperatures. The rate of decrease was similar for the two muscles. The decreased residual collagen was reflected by increased panel scores for tenderness.

Similar results were reported in another study by Ritchey and Hostetler (1964). As the end point temperature increased, panel scores indicated that connective tissue became softer and decreased in amount. Measured quantities of collagen nitrogen did not agree closely with panel scores for connective tissue. Within each treatment, three muscles from the round, semimembranosus, semitendinosus and biceps femoris, contained similar amounts of collagen nitrogen. This was not

reflected in panel scores. The pattern of connective tissue within the muscle structure might have influenced the scoring. For example, the biceps femoris had thick masses of connective tissue whereas the semitendinosus had finer strands.

Shimokomaki et al. (1972) pointed out that total collagen content is meaningless in explaining tenderness or lack of tenderness but that the relative proportion of thermally labile and thermally stable cross links is related to tenderness. Solubilization of collagen which reflects changes in cross links was studied.

In a study relating labile collagen from epimysial and intramuscular connective tissue to Warner Bratzler shear values on steaks taken from the same carcass, Field et al. (1970) found that connective tissue from the more tender longissimus muscle yielded a significantly higher amount of labile collagen than connective tissue from a less tender muscle, biceps femoris. Correlations between shear values of longissimus muscle and percent labile collagen approached significance. As shear values increased the percent of labile collagen decreased. The investigators concluded that within limits labile collagen is related to tenderness. The recognized limitation was that labile collagen in the range of 20-50 percent was characterized by low shear forces. Therefore, an increased yield of collagen within that range has little if any relationship to tenderness according to these investigators.

McClain et al. (1965a) elected to evaluate the relationship of the alkali insoluble collagen content of heated meat as well as that

of the raw meat to the tenderness of longissimus, semimembranosus and triceps brachii. Carcasses were divided into tender and less tender groups on the basis of shear values of the longissimus. Significant differences in collagen content among the three muscles or between the two tenderness groups were not found. Muscles tended to reach a relatively constant alkali-insoluble collagen content when heated regardless of initial content. The small, nonsignificant differences indicated a minor role for alkali-insoluble collagen in the tenderness of cooked meat. The absolute content of this collagen fraction was not related to shear values. The investigators suggested that the physical and the chemical state of the connective tissue and its role in the architectural aspects of muscle may influence tenderness.

Ringer insoluble and alkali insoluble collagen content of beef heated at two rates was studied by Bayne et al. (1971). Semimembranosus roasts were heated at 93°C and 149°C to an internal temperature of 70°C. Roasts cooked at 93°C were significantly more tender than the pair mates heated at 149°C. A significant decrease in alkali insoluble collagen content occurred in roasts cooked at both temperatures. However, neither the percent decrease nor the residual alkali insoluble content differed with respect to heating rate. Like results were reported for Ringer soluble and insoluble collagen. The collagen measurement provided no explanation for the difference in tenderness produced by the two heating rates.

The effect of time and temperature of heating on shear values and alkali insoluble collagen content of cores of beef biceps femoris

was investigated by Winstead (1970). Cores were heated at 55, 60 or 65°C for 30, 60 or 120 minutes. Cores heated at 60 and 65°C were more tender than those heated at 55°C. Cores heated for 120 minutes were more tender than those heated for 30 minutes regardless of temperature of heating. The effects of time and temperature were independent of each other. Residual collagen accounted for 53 percent of the increase in tenderness with heating.

In a similar study Chapman (1972) did not find a significant relationship between tenderness and alkali insoluble collagen content of semitendinosus cores heated at 60, 65 or 70°C for 60 or 180 minutes. Tenderness decreased as temperature increased and increased as time of heating increased. There is not universal agreement as to the role of alkali insoluble collagen in heated meat with regard to tenderness.

Paul et al. (1973) have shown a decrease in percent collagen solubilized in cores of beef heated at one rate to varying end point temperatures. Cores were heated in a water bath programmed to simulate oven roasting at 163°C (325°F). Correlations between shear values at each end point temperature and percent collagen solubilized were non-significant for semitendinosus muscle but significant for biceps femoris. Highly negative correlations between penetration data and percent collagen solubilized suggested that hardening of the muscle fibers was more important in tenderness changes than the breakdown of connective tissue with the heat treatment used in the study. Studies have not been reported that compare solubilization of collagen as reported by Paul et al. (1973) in meat heated at rates comparable to roasting at other oven temperatures.

IV. THE ROLE OF ENDOGENOUS ENZYMES IN THE TENDERIZATION OF BEEF

Increased tenderness of meat cooked at very low temperatures for long periods of time may be attributable partially to the action of enzymes that occur naturally in the muscle (Laakkonen et al., 1970b; Bayne et al., 1971). Little on the occurrence and activity of proteolytic enzymes in muscle tissues used as food was found in the literature. The role of enzymes in the tenderization of meat during post mortem aging is considered in reported studies.

Landmann (1963) noted that the cathepsins, a group of intracellular mammalian enzymes, have not been well characterized due to problems encountered in studying them. However, the investigator was able to isolate a proteinase system from beef muscle with an optimal pH range of 5 to 6 and concluded that if cathepsins were involved in tenderization during aging, conditions would be favorable to their activity.

Bodwell and Pearson (1964) prepared a partially purified bovine muscle cathepsin fraction and assayed its activity on a number of substrates. They concluded that the sarcoplasmic proteins are the major substrate for endogenous muscle cathepsins. Actin, myosin and actomyosin werenot affected by the enzyme fraction. Connective tissue or any of its components were not used as a substrate.

Proteolysis was unrelated to tenderization during post mortem aging (Davey and Gilbert, 1966). Changes in nonprotein nitrogen in longissimus dorsi aged at 2°C for 30 days were followed. Nonprotein

nitrogen was measured as a breakdown product of proteolytic action. Differences in rates of proteolysis among carcasses did not parallel differences in rates of tenderization.

The effect of pH, substrate concentration, incubation and temperature on the activity of cathepsins B, C and D isolated from bovine loin and flank, was evaluated by Lutalo-Bosa and MacRae (1969). The myofibrillar fractions of the muscles were used as the substrates. Connective tissue components were not subjected to the enzyme fraction. Optimal pH was 3.8 with significant activity shown at a pH of 4.8. Maximum activity was noted at 40°C with a rapid decrease in activity at higher temperatures. It was suggested that activity above 60°C was due to an enzyme known to be stable at 65°C with properties similar to those of cathepsin C. The investigators concluded that cathepsins B and D exhibited greater activity in muscle tissue than previously reported. Implications of the findings were not discussed.

The above studies have not considered the role of enzymes in tenderization during heating. Little work has been reported on proteolytic activity during the heating of meat.

Laakkonen et al. (1970b) reported detecting collagenolytic-like activity in the drip and a water soluble fraction of longissimus, biceps femoris and semitendinosus slices heated in plastic bags in a water bath. Slices were heated to 37, 45 and 60°C and held for a total of 10 hours heating time. The drip lost during heating exhibited more collagenolytic-like activity than the water soluble fraction from the heated meat. Therefore, it was concluded that in slow heating less

loss of drip would result in greater retention of enzymatic activity. Tests for general proteolytic activity confirmed the presence of other proteolytic enzymes. These enzymes were most active in meat heated to 37°C, significantly active in 45°C samples and clearly less active in samples heated to 58.5°C. In cooking methods involving a rapid rise in the temperature of meat to 70-80°C the enzymes would be inactivated. However, if the meat was slowly heated to 60°C, enzyme activity would be retained and could possibly be capable of producing tender meat. Although tenderness of the meat used in the enzyme studies was evaluated (Laakkonen et al., 1970a), no indication of the relationship between the enzyme activity and tenderness was reported.

The occurrence of collagenase in muscle tissues commonly used as food has not been reported. Collagenase is defined as an enzyme capable of cleaving molecules of native collagen. Cleavage must occur within the helical portion of the collagen molecule. Enzymes capable of attacking only those areas outside of the helix cannot be classified as collagenase (Seifter and Harper, 1971). However, the possible role of general proteolytic enzymes in tenderization should not be disregarded. Seifter and Harper (1970) noted that when the collagen molecule is denatured it becomes susceptible to general proteolytic attack.

CHAPTER III

PROCEDURE

I. SOURCE OF MEAT

One semitendinosus muscle from each of four Hereford X Charolais-Hereford (H X CH) and three Hereford X Charolais (H X C) heifers was secured from the Animal Science Department. Age of the animals ranged from 428 to 493 days. Mean U. S. D. A. quality grade for each breed group was high good (Winfree, 1973). The muscles were excised after 8 to 10 days aging in a cooler and placed in freezer storage until used in the study.

II. PREPARATION OF SAMPLES FOR HEATING

Each frozen semitendinosus muscle was cut into sections 2-1/4 inches long across the fibers. Epimysial connective tissue and adhering fat were removed while the muscle sections were allowed to thaw slightly to facilitate removal of 16 cores, 2-1/4 inches long and one inch in diameter. Cores were taken parallel to the muscle fibers and randomly assigned to heating rate-end point temperature treatments. Each heating rate-end point temperature combination will be referred to as a treatment for purposes of discussion. Two additional cores were taken and assigned to heating rates for thermocouples.

Cores were placed in weighed 50 milliliter Pyrex centrifuge tubes containing two small glass marbles and reweighed to determine raw core

weight. Marbles were used to keep the cores out of the drip as much as possible. A marble also was placed on top of each core to help keep the core in contact with the tube wall in order to maintain a uniform heat treatment. Cores were stored in the refrigerator for one or two days prior to heating.

The remaining muscle tissue was ground once through a plate having 10 mm holes and mixed well. Samples were taken for pH determinations and isolation of connective tissue.

Crude connective tissue (CCT) was isolated by the method of Field (1970). One hundred grams of ground muscle tissue were placed in a Waring Blendor with 400 ml of cold isotonic (0.9 percent) NaCl solution. The sample was blended at high speed for 10 seconds, placed in the refrigerator for 2 minutes and blended for an additional 5 seconds. The myofibrillar proteins and salt solution were allowed to pass through a single layer of cheesecloth. The residue was returned to the blendor and the procedure repeated four times. After the fifth blending, the remaining connective tissue was centrifuged at 4,000 x G for 5 minutes at 2-3°C. The supernatant was discarded. The CCT was placed on filter paper to remove excess moisture, weighed to determine yield and apportioned for heating.

III. HEATING

Tubes containing the cores of meat and tubes containing 0.97-1.80 g of CCT in 35 ml of 0.1 M potassium phosphate buffer, pH equivalent to the pH of the raw muscle, were placed in a shaker water bath

containing chilled water (6-12°C). Temperatures were monitored using two copper constantan thermocouples, one in a core of meat and one in a CCT-buffer tube. The temperature of the water bath was "programmed" to approximate the heat penetration curves obtained in a previous study (Smitherman, 1967). "Programming" was accomplished by adjusting the water bath temperature control every 8 minutes. The heating rates used were selected to simulate oven roasting of top round roasts ranging in weight from 1.9 to 2.3 kg at 93°C (200°F) for approximately 9-1/2 hours and at 149°C (300°F) for approximately 2-1/2 hours. The heating rates will be referred to as slow and fast, respectively. The order of heating, slow or fast, was randomized to minimize the effect of refrigerator storage of the cores.

Two tubes containing cores and two containing CCT-buffer samples were removed when corresponding tubes containing thermocouples reached temperatures of 40, 50, 60 and 70°C. Tubes were cooled in an ice bath for 15 minutes after removal from the water bath to terminate heating.

IV. METHODS OF EVALUATION

Following cooling, tubes containing cores were weighed to determine evaporative losses. Cores were removed from the tubes, adhering drip was removed and the cores were weighed. Cores were refrigerated overnight prior to further analysis.

Drip from the two cores from each heating rate-end point temperature treatment was combined. Weight of the drip was determined by

subtracting heated core weights and evaporative losses from the raw core weights. After homogenization at low speed for 30 seconds in a Virtis homogenizer, the drip was sampled for further analysis.

The buffers from each of the two CCT-buffer tubes from each treatment were combined in a mixing cylinder and made to volume of 80 ml with buffer prior to sampling for several analyses. The CCT samples were weighed, combined and sampled for hydroxyproline and guanidine hydrochloride soluble intramuscular collagen (GSIC) analysis.

Hydroxyproline Analysis

Changes in the connective tissue component of muscle tissue and in the isolated connective tissue were evaluated by Woessner's (1961) method for hydroxyproline. Prior to this analysis samples of appropriate weight or volume as listed in Table 1 were placed in culture tubes having screw caps. Each sample was made 6 N by the addition of hydrochloric acid. Volumes of acid and normality also are listed in Table 1. The samples were hydrolyzed in an autoclave at 121-122°C for 16-17 hours. Following hydrolysis a small amount of activated charcoal was added to tubes containing humin to facilitate decolorization. These tubes were placed on a shaker for 20 minutes and then filtered through Whatman No. 42 filter paper into a volumetric container of appropriate size. The filtrate was neutralized with concentrated sodium hydroxide using methyl red as an indicator and brought to volume with distilled water. The appropriate dilution volumes were determined in preliminary work and were dependent on the hydroxyproline content of the sample. Suitable volumes are listed in Table 1. Buffer and GSIC hydrolysates

Table 1. Hydroxyproline Analysis: Specifications for Sample Weights, Volumes and Dilutions

Type of Sample	Method Used[a]	Weight or Volume of Sample Hydrolyzed	HCl Added[b] (ml)	Normality of HCl	Volume of Diluted Hydrolysate (ml)	Sample Size for Analysis (ml)
Muscle	II	0.08-1.40 g	10.0	6.0	200 or 250	0.2-0.5
Drip	II	0.50-1.20 g	4.0	7.5	100 or 200	2.0
40° Water Soluble Fraction	II	5.00 ml	5.0	12.0	100	2.0
Buffer	I	2.50 ml	2.5	12.0	50 or 100	2.0
CCT	I	0.20-0.50 g	10.0	6.0	500	0.2-0.5
GSIC	I	2.50 ml	2.5	12.0	50	2.0

[a]Method I or II as outlined by Woessner, J. F. 1961. The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. Arch. Biochem. Biophys. 93: 440.

[b]Total volume for hydrolysis was 5 or 10 ml.

did not require decolorization with charcoal.

An aliquot of each diluted hydrolysate was pipetted into a test tube and the volume adjusted to 2 ml with water if necessary. Woessner (1961) outlined two methods for quantitative determination of hydroxyproline. Method I was used for samples containing at least 2 percent hydroxyproline and method II was used for samples containing a small amount of hydroxyproline in relation to other amino acids (Table 1). Details of each procedure are given in the Appendix.

Cores and Drip

Shear values. Following overnight refrigeration each core was sheared three times with a Warner Bratzler shear. Values for two cores from each treatment were averaged prior to statistical analysis. Cores from each treatment were ground once through a plate having 4 mm holes, mixed well and apportioned for analysis.

Moisture-fat determinations. Duplicate 3 to 5 g samples of ground muscle were weighed into preweighed Whatman extraction thimbles. Samples were dried in a vacuum oven at 60°C for 16 hours, weighed and extracted with petroleum ether (b.p. 37.4-50.0°C) for 6 hours on a Goldfisch Fat Extraction Apparatus. Following extraction samples were redried, weighed and percent nonfat-dry weight (NFDW) calculated.

Connective tissue solubilization in cores. A modification of the method described by Paul et al. (1973) was used to determine the amount of connective tissue solubilized during heating of the cores.

The term connective tissue solubilized rather than collagen solubilized is used since correction of hydroxyproline values and subsequent collagen values for elastin content was not made.

Five grams of muscle tissue from each treatment were homogenized with 50 ml of 40°C distilled water in a Waring Blendor for 2 minutes. The homogenate was transferred to a centrifuge tube and an additional 20-30 ml of water were used to rinse the blendor. The homogenate was centrifuged at 4,600 x G for 15 minutes. The supernatant was decanted through a single layer of cheesecloth into a mixing cylinder. The volume was recorded for use in calculating collagen content. Duplicate aliquots of the supernatant were analyzed for hydroxyproline content.

Duplicate weighed portions of the drip from each treatment were analyzed for hydroxyproline content. Hydroxyproline content of duplicate portions of each treatment sample of ground muscle also was determined.

Hydroxyproline values were converted to collagen values and percent connective tissue solubilized was calculated by the following equation:

$$\text{Percent Connective Tissue Solubilized} = \frac{\text{Collagen in Drip + Soluble Collagen in Muscle}}{\text{Collagen in Drip + Collagen in Muscle}} \times 100.$$

Isolation of water soluble fraction for proteolytic enzyme activity tests. A modification of the methods described by Laakkonen et al. (1970a) and Kronman et al. (1960) was used for the isolation of water soluble fractions for use in testing for proteolytic enzyme activity. Sixteen grams of raw, ground tissue in 50 ml of cold

demineralized water were stirred for one hour on a cold plate (2-3°C). Duplicate pH readings were taken on each sample immediately after stirring. The second pH measurement was preceded by restirring of the samples for 30 seconds and rotation of the flask (Rogers et al., 1967).

Each mixture then was centrifuged at 25,500 x G for one hour at 2-3°C. The supernatant was decanted through a single layer of cheesecloth into a mixing cylinder and brought to a volume of 64 ml with cold demineralized water. Sixteen grams of ground, heated muscle and one-half of the drip from each treatment were extracted as above and brought to volumes of 64 and 75 ml respectively. The pH of each heated sample also was determined as described previously.

Testing for proteolytic enzyme activity. Azocoll, an insoluble powdered cowhide-azo dye complex, was used as a substrate to test for the presence of proteolytic enzymes in the water soluble fraction of the raw and heated tissue as well as the drip. The azo dye is released from the collagen preparation by the action of proteolytic enzymes, the rate of release being indicative of the degree of proteolytic activity of the test material (Laakkonen et al., 1970b).

Six 10-ml portions of each water soluble fraction were added to 35 mg portions of Azocoll. Three tubes were incubated at 37°C for 15 minutes while the remaining three were kept at 0°C in an ice bath. Two blanks containing 35 mg of Azocoll and 10 ml of 0.1 M buffer, pH equivalent to the raw sample, also were prepared and incubated at 37°C.

Following incubation, proteolytic activity was terminated by filtration of the samples and blanks through Whatman No. 2 filter paper. Absorbance of each sample was measured in a Bausch and Lomb Spectronic 20. The difference between samples incubated at 37°C and an average of those incubated at 0°C and corrected with the blank value was calculated. To correct for differences in actual amount of nonfat dry matter in the muscle samples each absorbance value was divided by the percent nonfat-dry weight of the sample. Absorbance values of the drip samples were multiplied by two to give a value representative of the total drip. The adjusted absorbance values were termed the enzyme activity values and used in statistical analysis. No attempt was made to convert the absorbance values to specific enzyme activity units to avoid misleading interpretation and statements regarding the presence of specific enzymes. This type of information was not obtained in the present study.

Crude Connective Tissue-Buffer System

Connective tissue solubilization. An aliquot of the buffer and a portion of the CCT from each treatment were analyzed for hydroxyproline content (Woessner, 1961). Percent connective tissue solubilized during heating was calculated by the following equation:

$$\text{Percent Connective Tissue Solubilized} = \frac{\text{Collagen in Buffer}}{\text{Collagen in Buffer} + \text{Collagen in CCT}} \times 100 .$$

Peptide lengths. Peptide length of the solubilized collagen was estimated by measuring the terminal amino acid content of the buffer

with the 2,4,6-trinitrobenzenesulfonic acid (TNBS) method of Habeeb (1966) and total Kjeldahl (AOAC, 1970) nitrogen content of the buffer. A 2 ml aliquot of the heated buffer or a 1 ml aliquot plus 1 ml of unheated buffer was analyzed for terminal amino acid content. Two ml of a 4 g/100 ml solution of $NaHCO_3$ and 2 ml of an 0.1 g/100 ml solution of TNBS were added to the samples. The mixture was placed in a covered shaker water bath at 40°C for 2 hours. After the 2 hours, 2 ml of 1 N HCl were added and the absorbance read in a Bausch and Lomb Spectronic 20 at 410 nm against a buffer blank treated as above.

For each replication a solution of 1 μmole of glycine in 100 ml of buffer of the appropriate pH was prepared. Aliquots containing 0.1, 0.2, 0.3 and 0.4 μmoles of glycine were treated as above to obtain a standard curve. The slope of the curve was calculated by averaging the values $\frac{\mu\text{moles Glycine}}{\text{Absorbance}}$. This value was used in the following manner to calculate the terminal amino acid content in terms of μmoles glycine:

$$\frac{\mu\text{moles Glycine}}{\text{ml Buffer}} = \frac{\text{Absorbance of Sample}}{\text{ml Sample}} \times \text{Slope} .$$

The assumption that one μmole of glycine represents one μmole of free amino nitrogen was made.

Five ml aliquots of the heated buffers were analyzed for total nitrogen content by the micro-Kjeldahl method as outlined by AOAC (1970). Micro-moles of nitrogen per ml of sample were calculated according to the following equation:

$$\frac{\mu\text{moles N}}{\text{ml Sample}} = \frac{(\text{ml HCl for Sample} - \text{ml HCl for Blank})}{\text{ml Sample}} \times \text{Normality} \times 10^{-3}.$$

The following equation as suggested by Kang and Rice (1970) was used for estimating the length of the peptides released from CCT during heating:

$$\frac{\mu\text{moles Glycine/ml Sample}}{\mu\text{moles Nitrogen/ml Sample}} = \frac{1}{\text{Average Peptide Length}}.$$

Aldehyde content of connective tissue. Guanidine hydrochloride soluble intramuscular collagen (GSIC) was extracted from 1.9-2.4 g of CCT from each treatment. The CCT was extracted twice with 20 ml portions of 4 M guanidine hydrochloride for 24 hours in the cold. The second extraction was preceded by centrifugation at 2,000 x G at 2-3°C for 10 minutes. The combined supernatants were dialyzed against several changes of cold demineralized water for 7 days in the cold to precipitate the GSIC. The GSIC suspension was centrifuged at 8,200 x G for 20 minutes at 2-3°C, decanted and the precipitate resolubilized in 50 ml of 0.5 M acetic acid. To facilitate solubilization it was necessary to keep the GSIC-acetic acid mixture in the cold for several days. Intermittent shaking aided in the solubilization.

Aldehyde (α,β-unsaturated) content of the GSIC solution was determined according to the method of Levene (1962). Twelve ml of the solution were added to 4 ml of a saturated solution of 2,4-dinitrophenylhydrazine (2,4-DNPH) in 0.5 M acetic acid. The mixture was placed on a shaker at room temperature for 10 minutes and then incubated in a water bath at 40°C for 20 minutes. The solution then was dialyzed in the cold against several changes of 0.5 M acetic acid to remove excess

2,4-DNPH. A blank containing acetic acid in place of the 2,4-DNPH solution was prepared as above for each sample. Each sample was read in a Bausch and Lomb Spectronic 20 at 390 nm against its own blank. The equation for calculation of μmoles of aldehyde per μmole of collagen from the absorbance readings was:

$$\frac{\mu\text{moles Aldehyde}}{\mu\text{mole GSIC}} =$$

$$\frac{\text{O.D.} \times \text{Total Volume} \times \text{Molecular Wt. of Collagen} \times \frac{10^6\ \mu\text{moles}}{\text{mole}}}{\text{Extinction Coefficient of } \alpha,\beta\text{-unsaturated Aldehyde} \times \text{Length of Light Path} \times \frac{\text{mg GSIC}}{\text{ml Sample}} \times \text{Sample Volume}}$$

The molar extinction coefficient of 20,000 (Rójkind et al., 1964) for an α,β-unsaturated aldehyde was adjusted for the light path of the Bausch and Lomb Spectronic 20 by including the light path length (1.17 cm) in the equation. The molecular weight of collagen was given by Piez (1966) as somewhat less than 300,000. For purpose of this study a value of 300 mg of collagen per μmole was used.

Statistical Analysis

The experiment was set up in a completely randomized, split-plot design with seven blocks. Each muscle constituted one block or plot. Analysis of variance and orthogonal comparisons were used to study the functional relationship between heating rate and end point temperatures. When significance was found the Student-Newman-Keuls Test (Sokal and Rohlf, 1969) was applied. Shear data, connective tissue solubilization values, aldehyde content, peptide lengths and enzymatic activity values were evaluated in this manner. A correlation coefficient

was calculated to determine the relationship between shear values and percent solubilization of connective tissue.

CHAPTER IV

RESULTS AND DISCUSSION

I. HEATING RATES

The mean time-temperature curves for heating cores at two rates are shown in Figure 1. Mean total heating time for the slow method was 575 minutes, approximately 3-1/2 times the 169 minutes required to reach an internal temperature of 70°C with the fast rate of heating. Mean times to reach each of the end points in the cores and in the crude connective tissue-buffer system are both presented in Table 2. Heating time to each end point was longer for the slow rate than for the fast rate in both systems. Differences between the two systems in times required to reach the desired end points were apparently due to differences in the rate of heat transfer through the two media.

II. CHANGES IN CORES

Shear Values

Shear values for the seven replications of each treatment are shown in Table 3. Results of the analysis of variance of these values are presented in Table 4.

Shear values differed with respect to breed group. Cores from the H X C muscles were less tender ($P < 0.05$) than the cores from the H X CH muscles. Mean values for the two breed groups were 13.4 ± 1.3 and 11.9 ± 1.0 kg respectively.

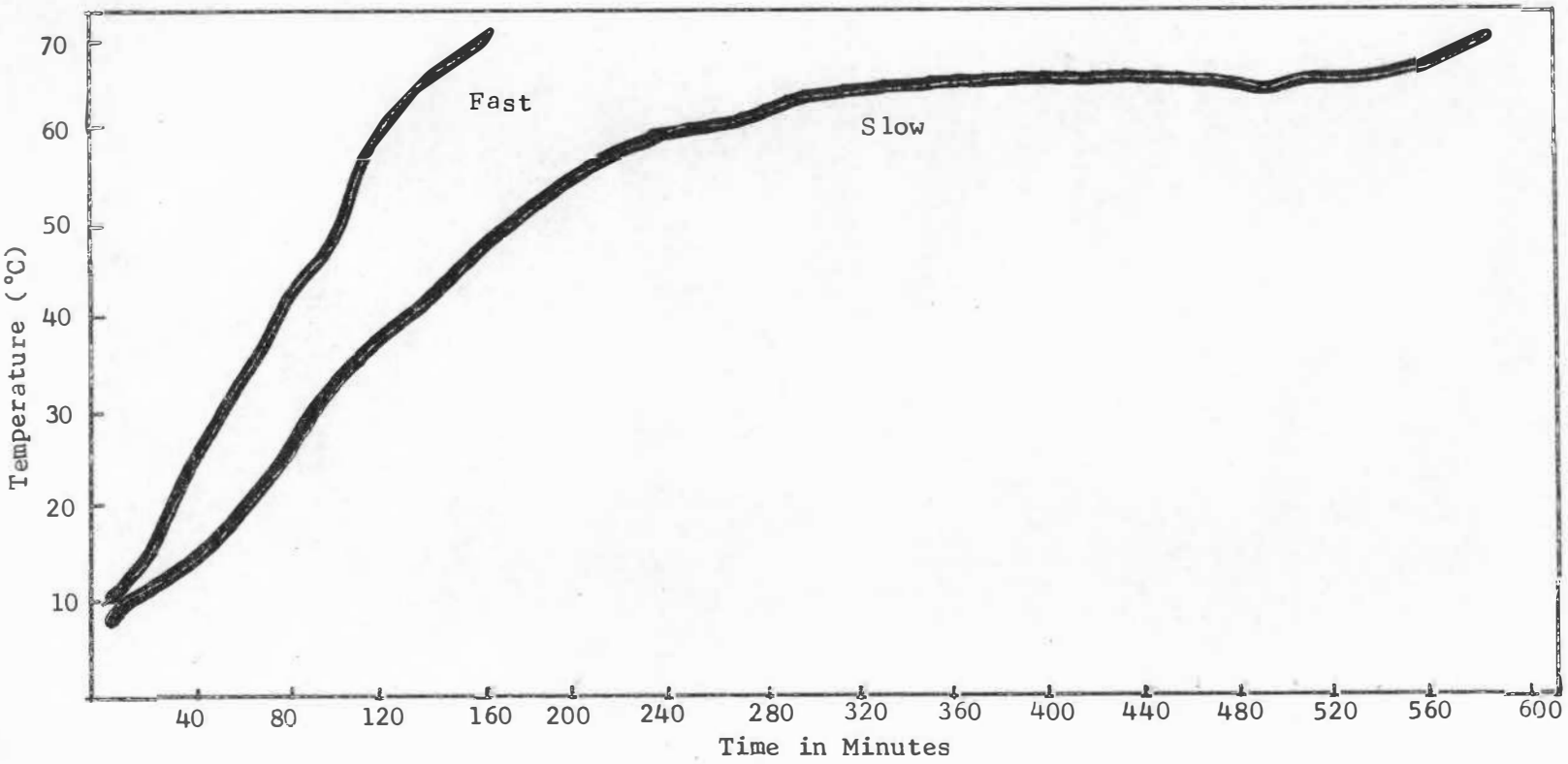

Figure 1. Time-Temperature Curves for Heating Semitendinosus Cores at Two Rates.

Table 2. Mean Minutes Required to Heat Semitendinosus Cores and Connective Tissue in Buffer at Two Rates to Four End Points

	Minutes[a]							
	Slow				Fast			
	40°C	50°C	60°C	70°C	40°C	50°C	60°C	70°C
Cores								
Mean	134	176	247	575	82	105	127	169
± Standard Error	3	1	5	7	2	2	2	2
Connective Tissue in Buffer								
Mean	123	165	212	546	75	93	115	150
± Standard Error	4	1	6	8	2	3	2	3

[a]Mean and standard error of seven replications.

Table 3. Shear Values of Semitendinosus Cores Heated at Two Rates to Four End Points

Breed	Muscle Number	Shear Values[a] (kg)							
		Slow				Fast			
		40°C	50°C	60°C	70°C	40°C	50°C	60°C	70°C
H X C	I	20.0	19.9	7.0	5.2	18.7	17.9	8.1	8.7
	III	18.9	19.0	7.7	5.6	21.4	19.8	7.6	7.6
	VII	22.4	19.5	7.0	6.1	21.5	18.0	6.8	7.3
H X CH	II	17.4	14.3	5.6	5.1	20.5	16.7	8.0	7.2
	IV	18.3	16.8	6.2	4.4	20.4	20.1	7.5	6.7
	V	18.0	18.6	6.8	4.5	17.6	17.2	7.5	8.1
	VI	17.7	16.0	5.3	5.4	15.1	15.1	6.5	6.4
Mean		19.0	17.7	6.5	5.2	19.3	17.8	7.4	7.4
± Standard Error		0.7	0.8	0.3	0.2	0.9	0.7	0.2	0.3

[a]Means of six shears.

Table 4. Mean Squares and F Values from Analysis of Variance of Shear Values of Heated Semitendinosus Cores

Source	Degrees of Freedom	Mean Squares	F Values
Rate	1	11.5	7.7**
End Point	3	657.1	438.1***
Linear	1	1700.8	1133.9***
Quadratic	1	1.6	1.1
Cubic	1	268.9	179.3***
Rate x End Point	3	3.2	2.1
Breed	1	30.8	12.3*
Rate x Breed	1	2.3	1.5
End Point x Breed	3	2.7	1.8
Rate x End Point x Breed	3	0.5	<1
Animal/Breed[a]	5	2.5	
Treatment x Animal/Breed[b]	35	1.5	

[a]Error term for breed.

[b]Error term for all sources except breed.

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Winfree (1973) reported a nonsignificant influence on Warner Bratzler shears of longissimus muscles from roasts and steaks from animals in these two breed groups. The muscles in the present investigation were taken from the animals used in the study by Winfree. Comparisons of the results of the two studies may be questioned, however, because different muscles and different rates of heating were used.

As expected, shear values of cores heated at the fast rate were higher ($P < 0.05$) than those heated at the slow rate. Respective mean shear values for the two rates were 13.0 ± 1.1 and 12.1 ± 1.2 kg. The findings for heating rate are in agreement with those of earlier workers (Bramblett et al., 1959; Bramblett and Vail, 1964; Bayne et al., 1969). The latter group of investigators reported that roasts heated to 67°C in a 93°C oven were more tender ($P < 0.001$) than those heated to 70°C in a 149°C oven.

Mean shear values of cores heated to four end point temperatures (T) without respect to heating rate are given in Table 5. The polynomial (curve A) presented in Figure 2 is representative of the end point shear values and was plotted from the following equation:

$$\text{Shear} = -463.7 + 28.318T - 0.538T^2 + 0.003T^3 .$$

As indicated in Table 4, shear values differed ($P < 0.001$) with respect to end point temperature. Results of the Student-Newman-Keuls Test appear in Table 5. A small but significant decrease in shear value occurred as cores were heated from 40 to 50°C. A greater decrease occurred in the 50 to 60°C interval. Heating above 60°C did not significantly affect tenderness.

Table 5. Mean Shear Values and Percent Solubilized Connective Tissue Values of Semitendinosus Cores Heated to Four End Points

End Point (°C)	Mean Shear Value[a] (kg)	Solubilized Connective Tissue[a] (%)
40	$19.1^{b} \pm 0.5$	$1.62^{b} \pm 0.41$
50	$17.8^{c} \pm 0.5$	$2.69^{b} \pm 0.28$
60	$7.0^{d} \pm 0.2$	$5.63^{c} \pm 0.61$
70	$6.3^{d} \pm 0.4$	$11.23^{d} \pm 1.04$

[a]Mean and standard error of seven replications.

[b,c,d]Means in the same column with like superscripts do not differ ($P < 0.01$).

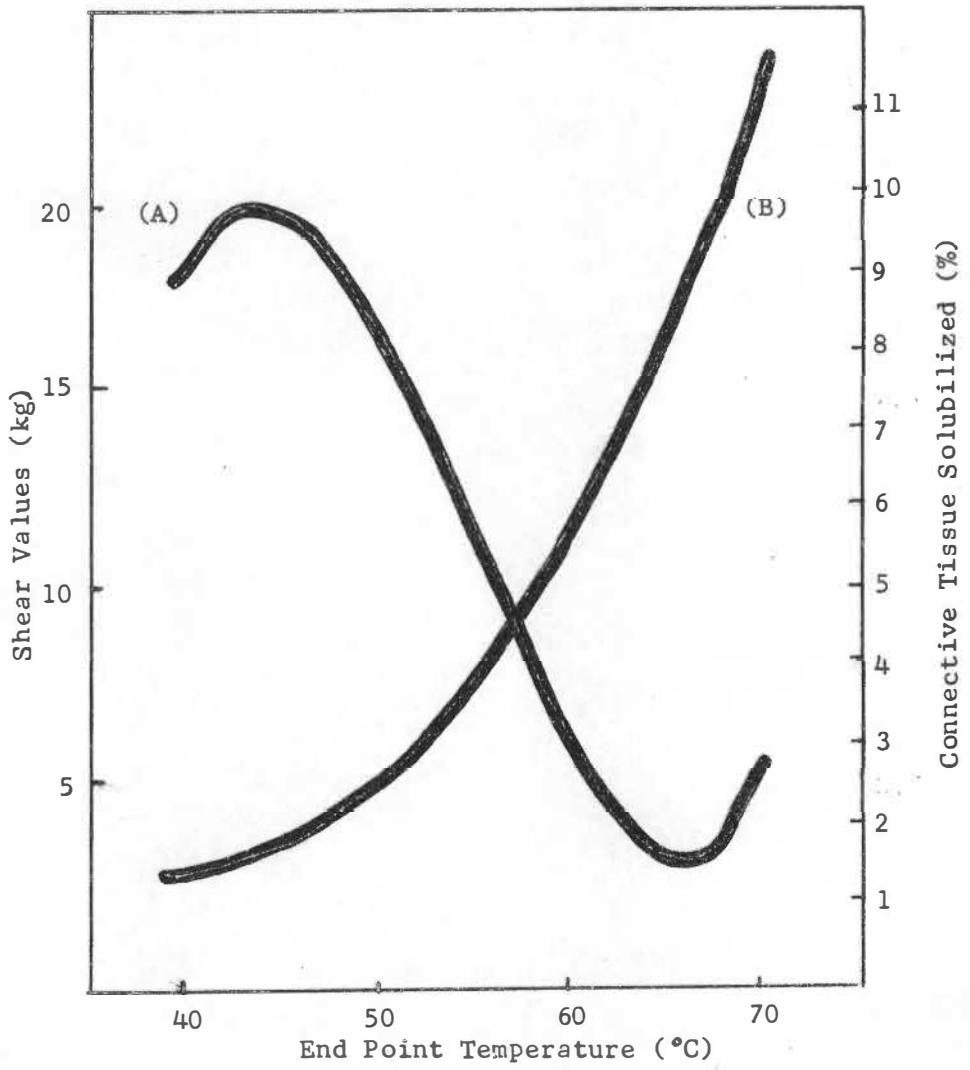

Figure 2. Shear Values (A) and Percent Connective Tissue Solubilized (B) as a Function of End Point Temperature.

Tenderness of semitendinosus muscle was associated with end point in several earlier studies. Sartorius and Child (1938) noted an increase in tenderness with heating to 58 and 67°C and a decrease in tenderness at 75°C. Laakkonen et al. (1970a) found that the major decrease in shear values of semitendinosus muscle heated at a slow rate occurred as the meat warmed from 50 to 60°C. On the other hand, Paul et al. (1973) reported that end point did not influence tenderness of semitendinosus cores. A decrease in tenderness with heating in the range 56 to 59°C as a result of collagen shrinkage was reported (Machlik and Draudt, 1963).

The effect of heating rate was independent of the effect of end point temperature (Table 4, page 39). However, the treatment means (Table 3, page 38) suggest a trend toward increased tenderness between 60 and 70°C at the slower rate of heating. The difference between the 70°C cores heated at the two rates is similar to the difference reported by Bayne et al. (1969) in which shear values for large, top round roasts heated at 149 and 93°C were 8.5 and 7.9 kg respectively.

Percent Connective Tissue Solubilized

Values for connective tissue solubilization are shown in Table 6. As with the shear values, heating rate had a significant ($P < 0.01$) influence on percent solubilized connective tissue (Table 7). At the slower rate without respect to end point, 5.92 $\pm$ 0.96 percent of the connective tissue was solubilized. This was significantly greater than the 4.67 $\pm$ 0.70 percent solubilized at the fast rate.

Table 6. Percent Connective Tissue Solubilized in Semitendinosus Cores Heated at Two Rates to Four End Points

Breed	Muscle Number	Connective Tissue Solubilized (%)							
		Slow				Fast			
		40°C	50°C	60°C	70°C	40°C	50°C	60°C	70°C
H X C	I	4.87	4.61	9.28	19.59	0.31	3.61	9.58	15.29
	III	4.16	3.17	5.07	14.35	0.90	3.30	4.00	7.02
	VII	1.64	3.74	5.84	11.24	0.68	2.87	5.60	8.16
H X CH	II	2.04	1.80	7.02	13.84	2.99	3.15	9.11	12.48
	IV	0.44	1.07	4.33	11.09	2.81	2.87	3.46	7.15
	V	0.35	2.62	5.14	13.22	0.22	1.98	2.60	6.48
	VI	0.15	0.80	3.46	10.69	1.05	2.09	4.29	6.62
Mean		1.95[a]	2.54[a]	5.73[b]	13.43[c]	1.28[a]	2.84[a]	5.52[b]	9.03[d]
± Standard Error		0.72	0.53	0.73	1.16	0.43	0.23	1.05	1.31

[a,b,c,d]Means with like superscripts do not differ ($P < 0.01$).

Table 7. Mean Squares and F Values from Analysis of Variance of Percent Connective Tissue Solubilized in Heated Semitendinosus Cores

Source	Degrees of Freedom	Mean Squares	F Values
Rate	1	21.81	8.94**
End Point	3	259.73	106.45***
Linear	1	707.01	289.76***
Quadratic	1	71.71	29.31**
Cubic	1	0.46	<1
Rate x End Point	3	16.02	6.56**
Breed	1	34.97	1.76
Rate x Breed	1	9.25	3.79
End Point x Breed	3	1.43	<1
Rate x End Point x Breed	3	2.27	<1
Animal/Breed[a]	5	19.89	
Treatment x Animal/Breed[b]	35	2.44	

[a]Error term for breed.

[b]Error term for all sources except breed.

**$P < 0.01$; ***$P < 0.001$.

Bayne et al. (1971) found that percent alkali insoluble collagen solubilized with heating did not differ with respect to heating rate. Data were obtained by difference (i.e., raw insoluble collagen minus heated insoluble collagen). Therefore, total collagen was not considered as in the present study.

As end point temperature increased, a significantly ($P < 0.001$) greater portion of the connective tissue was solubilized. End point means for solubilized connective tissue are presented in Table 5, page 41. The polynomial representing the best fit curve for these data are shown in Figure 2, curve B, page 42. The equation for the curve is:

$$\text{Percent Connective Tissue Solubilized} = 20.662 - 0.928T + 0.011T^2 .$$

A small nonsignificant increase occurred from 40° to 50°C. Significant increases in solubilized connective tissue occurred from 50° to 60°C and from 60° to 70°C (Table 5 and Figure 2).

An increased ($P < 0.01$) solubilization of collagen in semitendinosus with increasing internal temperature was reported by Paul et al. (1973). Direct comparison of the results would be impossible for several reasons. The two investigations differed with respect to heating rates and end points. Paul and co-workers corrected hydroxyproline data for elastin content. This was not done in the present study. Elastin contains 1-2 percent as much hydroxyproline as collagen (Paul, 1972). It is not affected by heat and it is a major component of connective tissue of the semitendinosus muscle, representing approximately 37 percent of the total connective tissue (Bendall, 1967).

Bendall suggested that elastin affects tenderness of heated meat in a manner similar to that of denatured collagen. Therefore, the correction for elastin content was not made.

The effect of end point temperature was related to the rate of heating as shown in the analysis of variance (Table 7, page 45). Results of the Student-Newman-Keuls Test for treatment means appear in Table 6, page 44. A greater ($P < 0.01$) percentage of connective tissue was solubilized in cores heated at the slow rate to 70°C than in those heated to 70°C at the fast rate. Differences among 40, 50 and 60°C samples with respect to heating rate were nonsignificant. Differences within heating rates with respect to end point temperatures were identical to the differences in end point means described previously (Table 5, page 41).

Relationship Between Connective Tissue Solubilization and Shear Values

A significant relationship between shear values and percent connective tissue solubilized during heating was found. As percent connective tissue solubilized increased shear values decreased ($r = -0.704$; $P < 0.01$). The relationship is illustrated in Figure 2, page 42. There appeared to be a limit to this relationship, however. The small but significant ($P < 0.01$) decrease in shear value from 40 to 50°C was not paralleled by a significant increase in connective tissue solubilization at the slow or the fast rate (Table 5 and Table 6). As a significant increase in percent solubilization of connective tissue occurred from 50 to 60°C a significant decrease in shear values was

found. The significant difference in percent solubilized connective tissue in the slow 70°C and the fast 70°C samples was not reflected in a statistically significant difference in shear values between the two samples.

The increased solubilization of connective tissue in the slow 70°C cores might have been overshadowed by other factors important in the determination of tenderness such as hardening of the myofibrillar proteins during the long period of heating from 60 to 70°C. After comparing shear values and penetration data of semitendinosus strips, Paul et al. (1973) concluded that connective tissue breakdown was less important than muscle fiber coagulation in control of tenderness changes in the strips heated to 82°C. Lack of correlation between percent solubilized connective tissue and shear values was reported. Differences in the end point temperatures studied might be partially responsible for the differences in the results of the reported study and the present investigation. Draudt (1972) implied that hardening of the myofibrillar proteins occurred between 60 and 74°C. Heating above 70°C as in the study of Paul et al. (1973) might have resulted in greater hardening than was seen in the present study.

Limitations in the relationship between tenderness and labile collagen were recognized by Field et al. (1970). At low shear values, increased labile collagen has little effect on tenderness.

The effects of heating rate and end point temperatures on shear values cannot be completely explained in terms of percent connective tissue solubilized.

Moisture-Fat and pH Values

Percent nonfat dry weight increased with heating. Data for the seven replications are reported in Table 20 (Appendix). These values were not analyzed statistically.

An increase in pH values occurred with heating but was not evaluated statistically. Values for pH of raw muscle samples ranged from 5.57 to 5.74 with a mean of 5.64 $\pm$ 0.3. Samples heated at the slow rate increased to pH 5.84 $\pm$ 0.1 whereas the fast samples increased to pH 5.81 $\pm$ 0.1. Complete pH data are presented in Table 21 (Appendix).

Proteolytic Enzyme Activity

Values for proteolytic activity of muscle samples and for the drip lost during heating are presented in Tables 8 and 9.

Water soluble extracts of the raw muscle samples were tested for proteolytic activity. Values are included in Table 8. The raw meat values were not included in the statistical analysis. However, inspection of the data shows that the mean raw meat value is greater than the means for any of the treatment samples. There are a few heated values that are greater than the lower raw values.

Analysis of variance (Table 10) of the enzyme activity values (EAV) indicated that end point temperature had a significant ($P < 0.05$) influence on the level of enzyme activity of the water soluble fraction of the heated cores. End point means are presented in Table 11. A significant curvilinear trend was found in these data. The polynomial in Figure 3 (curve A) illustrates this trend and was plotted from the equation:

Table 8. Proteolytic Enzyme Activity of Raw Semitendinosus Muscle and Semitendinosus Cores Heated at Two Rates to Four End Points

Breed	Muscle Number	Enzyme Activity Value[a]								
			Slow				Fast			
		Raw	40°C	50°C	60°C	70°C	40°C	50°C	60°C	70°C
H X C	I	0.159	0.052	0.000	0.131	0.029	0.035	0.055	0.089	0.030
	III	0.123	0.083	0.071	0.111	0.058	0.128	0.072	0.084	0.019
	VII	0.372	0.082	0.070	0.056	0.029	0.110	0.097	0.000	0.012
H X CH	II	0.143	0.117	0.140	0.080	0.028	0.086	0.081	0.191	0.073
	IV	0.338	0.071	0.081	0.078	0.081	0.212	0.140	0.172	0.077
	V	0.249	0.090	0.085	0.125	0.139	0.090	0.078	0.062	0.003
	VI	0.192	0.019	0.049	0.097	0.055	0.078	0.086	0.089	0.053
Mean		0.225	0.073	0.071	0.097	0.060	0.106	0.087	0.098	0.038
± Standard Error		0.031	0.010	0.014	0.010	0.014	0.020	0.010	0.022	0.010

[a] $\frac{\text{Absorbance}}{\% \text{ NFDW}}$.

Table 9. Proteolytic Enzyme Activity of Drip Lost from Semitendinosus Cores Heated at Two Rates to Four End Points

Breed	Muscle Number	Enzyme Activity Value[a]							
		Slow				Fast			
		40°C	50°C	60°C	70°C	40°C	50°C	60°C	70°C
H X C	I	0.000	0.000	0.000	0.000	0.000	0.008	0.006	0.000
	III	0.040	0.020	0.030	0.008	0.018	0.008	0.000	0.010
	VII	0.036	0.020	0.010	0.002	0.032	0.022	0.000	0.002
H X CH	II	0.022	0.022	0.010	0.008	0.000	0.000	0.000	0.000
	IV	0.026	0.032	0.024	0.022	0.066	0.014	0.002	0.014
	V	0.034	0.006	0.016	0.000	0.008	0.008	0.004	0.000
	VI	0.016	0.018	0.000	0.008	0.016	0.000	0.002	0.000
Mean		0.025	0.017	0.013	0.007	0.020	0.008	0.002	0.005
± Standard Error		0.005	0.004	0.004	0.003	0.009	0.003	0.001	0.002

[a]Mean value (absorbance x 2) of triplicate determinations from one water soluble extract.

Table 10. Mean Squares and F Values from Analysis of Variance of Proteolytic Enzyme Activity of Heated Semitendinosus Cores and of Drip Lost During Heating

Source	Degrees of Freedom	Enzyme Activity of Muscle		Enzyme Activity of Drip	
		Mean Squares	F Values	Mean Squares	F Values
Rate	1	0.0007	<1	0.00058	5.27*
End Point	3	0.0063	4.20*	0.00077	7.00***
Linear	1	0.0074	4.93**	0.00208	18.91***
Quadratic	1	0.0050	3.33	0.00024	2.18
Cubic	1	0.0065	4.33**	0.00000	0
Rate x End Point	3	0.0018	1.20	0.00006	<1
Breed	1	0.0109	4.04	0.00002	<1
Rate x Breed	1	0.0011	<1	0.00002	<1
End Point x Breed	3	0.0010	<1	0.00002	<1
Rate x End Point x Breed	3	0.0015	1	0.00006	<1
Animal/Breed[a]	5	0.0027		0.00055	
Treatment x Animal/Breed[b]	35	0.0015		0.00011	

[a]Error term for breed.

[b]Error term for all sources except breed.

*P < 0.05; **P< 0.01; ***P< 0.001.

Table 11. Mean Proteolytic Enzyme Activity of Heated Semitendinosus Cores and of Drip Lost During Heating to Four End Points

End Point (°C)	Enzyme Activity[a]	
	Cores[b]	Drip[c]
40	$0.090^{d} \pm 0.010$	$0.022^{f} \pm 0.005$
50	$0.079^{d} \pm 0.009$	$0.013^{f,g} \pm 0.003$
60	$0.098^{d} \pm 0.010$	$0.007^{g} \pm 0.003$
70	$0.049^{e} \pm 0.010$	$0.006^{g} \pm 0.002$

[a] Means and standard errors of seven replications.

[b] $\frac{\text{Absorbance}}{\% \text{ NFDW}}$.

[c] Absorbance.

[d,e] Means with like superscripts do not differ ($P < 0.05$).

[f,g] Means with like superscripts do not differ ($P < 0.01$).

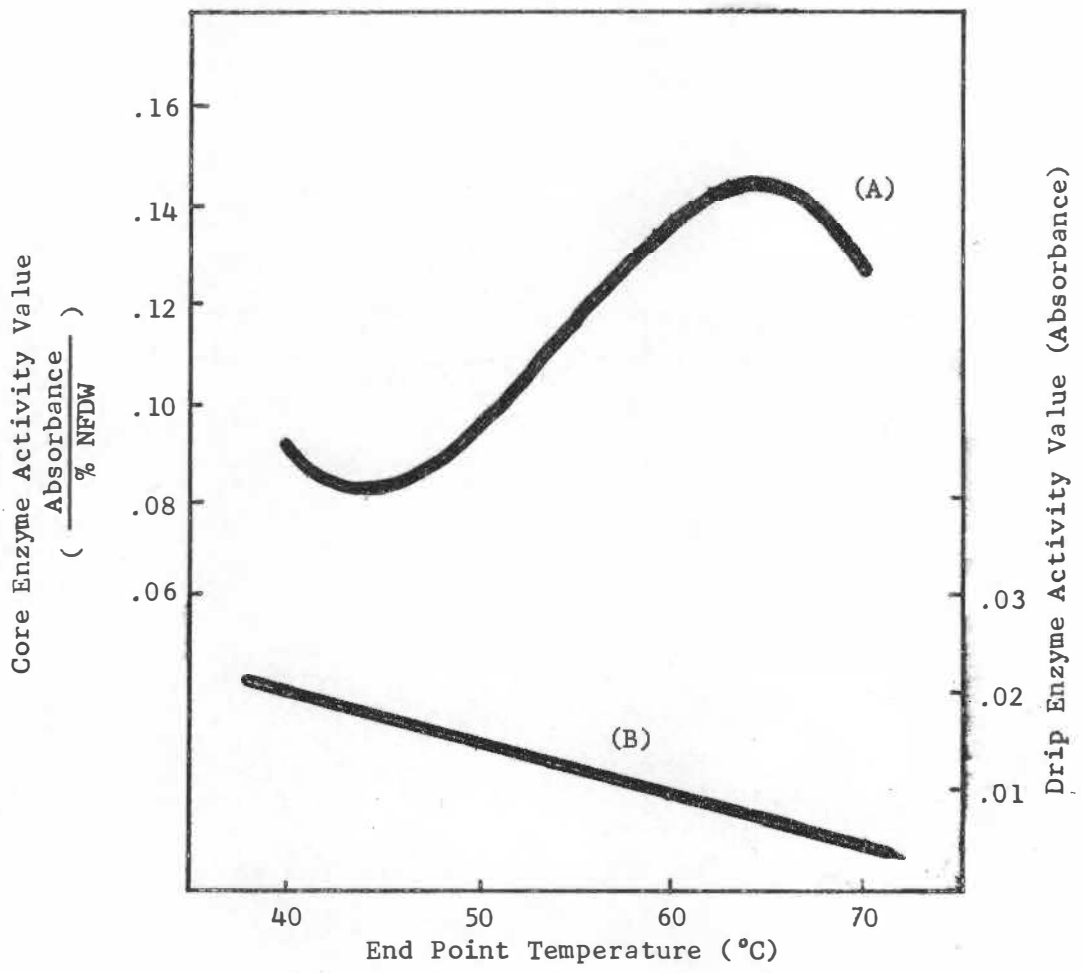

Figure 3. Proteolytic Enzyme Activity of Cores (A) and Drip (B) as a Function of End Point Temperature.

$$EAV_{core} = 2.394 - 0.136T - 0.0026T^2 - 0.000016T^3 .$$

A significant change in activity was not seen as the cores were heated from 40 to 60°C. A significant ($P < 0.05$) decrease occurred between 60 and 70°C. A limited number of beef muscles was tested by Laakkonen et al. (1970b) and an increase in proteolytic activity with heating to 50.5°C was reported.

Rate of heating did not affect the level of enzyme activity. The effect of the end point temperature on activity was independent of the other factors in this study including heating rate.

A different pattern of proteolytic activity was exhibited by the drip samples. A significant ($P < 0.001$) linear decrease (Table 10, page 52) in activity in the drip was shown. Curve B in Figure 3 represents the equation:

$$EAV_{drip} = 0.0417 - 0.00054T .$$

As end point temperature increased from 40 to 60°C a significant decrease in activity occurred. Laakkonen et al. (1970b) noted that drip values changed in the same manner as muscle sample values with heating.

Drip lost from slowly heated cores exhibited greater ($P < 0.05$) proteolytic activity than the drip from cores heated at the faster rate. Respective mean activity values were 0.015 ± 0.002 and 0.009 ± 0.003. The effects of heating rate and end point temperatures on the proteolytic activity exhibited by the drip were independent of each other.

From these data it is evident that general proteolytic enzymes could be active during heating of meat. Landmann (1963) noted that the optimal pH for a proteinase system isolated from beef muscle was in the

range pH 5-6. All muscle samples in this study were within this range. Although it has been reported (Bodwell and Pearson, 1964) that the sarcoplasmic proteins are the major substrate for endogenous muscle cathepsins, it also was reported that denatured collagen is susceptible to general proteolytic action (Seifter and Harper, 1970). If the collagen of muscle tissue is denatured prior to inactivation of proteolytic enzymes during heating, degradation of the collagen molecule could result. This might affect the tenderness of the meat. The degree of breakage of peptide bonds necessary to affect tenderness of meat has not been established.

Differences in tenderness attributable to heating rate may partially result from differing degrees of enzymatic breakdown of collagen. The greatest decrease in shear values occurred between 50 and 60°C (Table 5, page 41). This decrease in shear values was paralleled by an increase in enzymatic activity (Table 11, page 53). The time in this temperature range was greater for slowly heated cores (Table 2, page 37). Therefore, an opportunity for prolonged enzymatic activity in the slowly heated samples could result in differences in the breakdown of collagen. The trend shown in Figure 3 suggested that more than one proteolytic enzyme system might have been operative in the muscle samples. One system was inactivated during heating to approximately 44°C. A second system increased in activity to a maximum value at 66°C followed by a gradual decrease in activity. Lutalo-Bosa and MacRae (1969) observed proteolytic activity in beef muscle extracts incubated above 60°C and suggested that an enzyme similar to

cathepsin C which is heat stable at 65°C was responsible. Other cathepsins are inactivated above 60°C. The possibility that enzymatic activity during the long period of heating from 60 to 70°C resulted in increased connective tissue solubilization in slow 70°C samples (Table 6, page 44) was not disproved in the present investigation.

III. CHANGES IN CONNECTIVE TISSUE HEATED IN BUFFER

Isolation of Connective Tissue

Yield of crude connective tissue isolated from ground, raw muscle ranged from 4.49 to 7.74 percent on a wet basis. The mean yield was 5.54 $\pm$ 0.41 percent. Field (1970) reported a yield of 3.88 percent from longissimus dorsi. The greater yield obtained in the present study was expected because of the difference in collagen and elastin content of the two muscles.

Solubilization of Connective Tissue

The percentage of connective tissue solubilized during heating in buffer is shown in Table 12. Little or no solubilization was evident in the 40 and 50°C samples. Evidently soluble hydroxyproline containing materials that were found in the intact cores heated to these two temperatures were removed in the process of isolating the connective tissue from other components of the muscle tissue. Or, a nonheat related reaction was affecting solubilization of collagen from the beginning of the heating period to 50°C in the intact meat system. Enzyme action might be a possibility.

Table 12. Percent Connective Tissue Solubilized During Heating in Buffer at Two Rates to Four End Points

Breed	Muscle Number	Connective Tissue Solubilized (%)							
		Slow				Fast			
		40°C	50°C	60°C	70°C	40°C	50°C	60°C	70°C
H X C	I	0.00	0.10	2.25	12.96	0.00	0.00	2.52	4.81
	III	0.00	0.00	1.80	11.05	0.00	0.00	0.76	3.81
	VII	0.00	0.00	4.77	13.64	0.00	0.00	2.58	6.66
H X CH	II	0.00	0.00	2.58	17.25	0.00	0.00	1.39	4.30
	IV	0.00	0.00	3.15	9.61	0.00	0.00	1.44	5.75
	V	0.00	0.24	2.38	10.50	0.00	0.00	1.33	6.16
	VI	0.00	0.00	2.67	10.08	0.00	0.00	1.81	4.66
Mean		0.00	0.05	2.80	12.16	0.00	0.00	1.69	5.16
± Standard Error		0.00	0.03	0.36	1.02	0.00	0.00	0.25	0.39

The 40 and 50°C samples were not considered in the analysis of variance. The results of the analysis for the 60 and 70°C sample values are reported in Table 13. Significant differences ($P < 0.001$) with respect to heating rate and end point temperatures were found. Solubilization of 7.48 ± 1.40 percent connective tissue at the slow rate without respect to end point was significantly greater than the 3.43 ± 0.53 percent solubilized in samples heated at the fast rate. Heating to 70°C at both rates solubilized 8.66 ± 1.10 percent of the connective tissue as compared to 2.24 ± 0.26 percent in the 60°C samples.

The effects of heating rate and end point temperature on connective tissue solubilization were interrelated. Treatment means reflecting this interaction ($P < 0.01$) are given in Table 14. The slow and fast 60°C samples did not differ but were significantly lower than the slow and fast 70°C samples. Significantly more connective tissue was solubilized at 70°C in the slow samples than in the fast samples.

A comparison of these results with the results of the Student-Newman-Keuls test of treatment means in Table 6, page 44, indicated that the pattern of solubilization of connective tissue in the intact cores was similar to that in the buffer connective tissue system at the higher two end points. Therefore, results of peptide and aldehyde studies might give some insight into the changes occurring in connective tissue at the upper end of the heating curve.

Peptide Length Estimations

Estimated lengths of the peptides released with heating of the CCT-buffer systems are listed in Table 15. End point temperature was

Table 13. Mean Squares and F Values from Analysis of Variance of Values Characterizing Connective Tissue Changes During Heating in Buffer: Percent Connective Tissue Solubilized, Peptide Lengths and Aldehyde Content

Source	Degrees of Freedom	% Solubilized		Peptide Length		Aldehyde Content	
		Mean Squares	F Values	Mean Squares	F Values	Mean Squares	F Values
Rate	1	114.86	45.76***	3.48	<1	93.06	4.08+
End Point	3[a]	288.07	114.77***	108.92	3.99*	62.68	2.75+
Linear	1	--	--	166.94	6.12**	--	--
Quadratic	1	--	--	152.79	5.60**	--	--
Cubic	1	--	--	7.04	<1	--	--
Rate x End Point	3[a]	60.53	24.11***	20.46	<1	26.54	1.16
Breed	1	0.70	<1	73.46	<1	164.06	<1
Rate x Breed	1	0.15	<1	1.42	<1	17.55	<1
End Point x Breed	3[a]	0.00	<1	15.60	<1	22.04	<1
Rate x End Point x Breed	3[a]	0.46	<1	0.12	<1	6.05	<1
Animal/Breed[c]	5	3.86		89.67		219.65	
Treatment x Animal Breed[d]	35[b]	3.51		27.29		22.82	

[a]One for percent solubilized connective tissue since only 60 and 70°C values were included.

[b]Fifteen for percent solubilized connective tissue since only 60 and 70°C values were included.

[c]Error term for breed.

[d]Error term for all sources except breed.

+$0.10 > P > 0.05$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 14. Mean Percent Connective Tissue Solubilized During Heating in Buffer at Two Rates to Two End Points

Treatment		Solubilized Connective Tissue[a] (%)
Rate	End Point (°C)	
Slow	60	$2.80^{b} \pm 0.36$
Slow	70	$12.16^{d} \pm 1.02$
Fast	60	$1.69^{b} \pm 0.25$
Fast	70	$5.16^{c} \pm 0.39$

[a]Means and standard errors of seven replications.

[b,c,d]Means with like superscripts do not differ ($P < 0.01$).

Table 15. Length of Peptides Released from Connective Tissue Heated in Buffer at Two Rates to Four End Points

Breed	Muscle Number	Estimated Average Peptide Chain Length (Number of Amino Acids)							
		Slow				Fast			
		40°C	50°C	60°C	70°C	40°C	50°C	60°C	70°C
H X C	I	7.38	10.90	14.99	34.06	24.49	20.36	16.89	23.77
	III	14.33	12.57	16.39	18.25	9.03	10.56	10.50	15.88
	VII	30.95	18.05	17.54	16.60	25.82	18.04	16.40	21.84
H X CH	II	8.35	8.04	12.41	18.35	5.07	9.96	9.78	15.80
	IV	18.29	12.83	15.69	21.57	14.18	19.37	19.66	17.64
	V	12.79	10.01	12.15	24.42	17.30	15.36	16.46	28.37
	VI	8.59	19.18	21.05	16.78	21.65	17.24	10.86	14.19
Mean		14.38	13.08	15.75	21.43	16.79	15.84	14.36	19.64
± Standard Error		3.13	1.56	1.16	2.36	2.96	1.56	1.47	1.96

the only factor studied that had an effect on the length of peptide released. Those released during heating to 70°C were significantly longer than the peptides released in samples heated to 60°C and below (Table 16). A significant curvilinear trend in these data was noted and is illustrated in Figure 4. The polynomial was plotted from the following equation:

$$\text{Peptide Length} = 55.838 - 1.663T + 0.016T^2.$$

The 40, 50 and 60°C samples contained peptides of similar lengths. This suggested that the same bonds were affected by the heating process. Goll et al. (1964b) made a like observation in samples of lyophilized collagen heated to 70 and 100°C. It then follows that heat may affect different bonds above 60°C. This difference may be related to the collagen shrinkage reaction. The collagen shrinkage temperature of beef has been listed by several (Machlik and Draudt, 1963; Hamm, 1966) as 63°C. Goll et al. (1964b) noted that the thermal shrinkage temperature was that temperature at which a sudden release of hydroxyproline occurred. This release could be responsible for the increased solubilization of connective tissue from 60 to 70°C as well as the change in average peptide length.

A discrepancy in these data and percent connective tissue solubilized must be noted. Nitrogen containing materials released from the connective tissue during the early stages of heating (i.e., to 40 and 50°C) were not collagenous in nature since hydroxyproline was not detected in these samples (Table 12, page 58). Since samples at 60 and 70°C were not corrected for these non-collagenous peptides they must be

Table 16. Mean Estimated Lengths of Peptides Released from Connective Tissue Heated in Buffer to Four End Points

End Point (°C)	Mean Estimated Peptide Length[a] (No. of Amino Acids)
40	$15.59^{b} \pm 2.10$
50	$14.46^{b} \pm 1.23$
60	$15.06^{b} \pm 0.92$
70	$20.54^{c} \pm 1.49$

[a]Mean and standard error of seven replications.

[b,c]Means with like superscripts do not differ ($P < 0.05$).

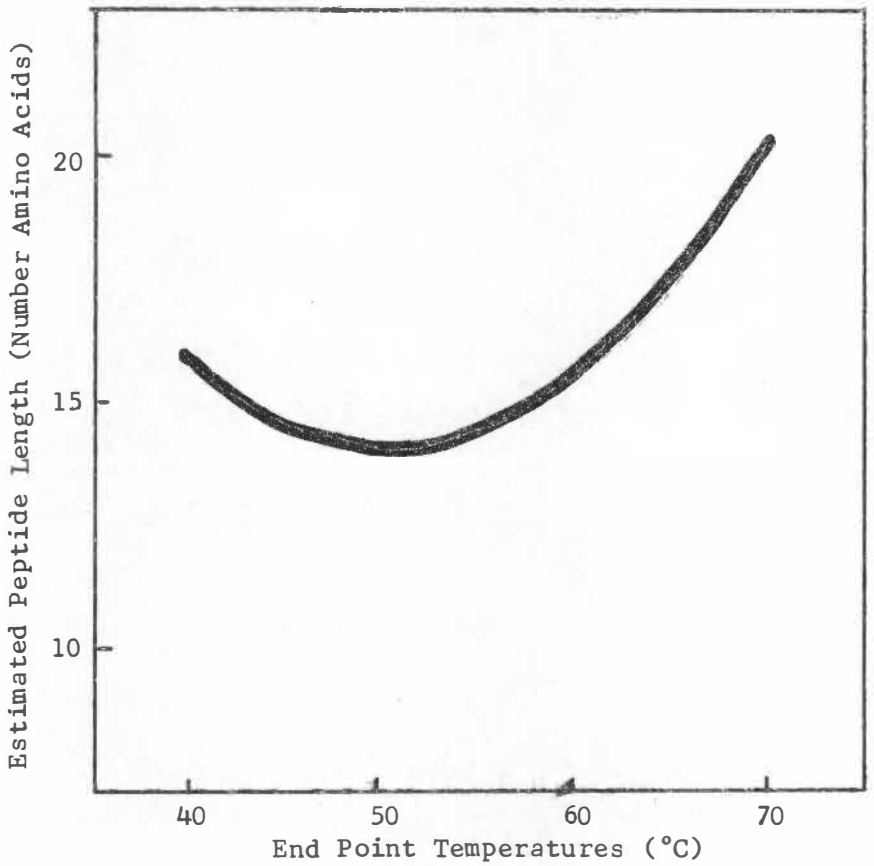

Figure 4. Estimated Length of Peptides Released from Crude Connective Tissue During Heating in Buffer as a Function of End Point Temperature.

treated as estimates as indicated. They do not give a true estimate of the length of hydroxyproline containing peptides. They do give a general indication of the changes occurring, however.

Aldehyde Content

Data for aldehyde content were evaluated with the analysis of variance as planned. Results are shown in Table 13, page 60. Large sums of squares for Animal/Breed and Treatment x Animal/Breed were indicative of considerable variation in the data in both animal variation and experimental error. This also was reflected in the standard errors of the means (Table 22, Appendix). Because of the large variation no attempt to relate these changes to the possible heat effects on cross linking of collagen was made.

Differences in the amount of guanidine hydrochloride soluble intramuscular collagen (GSIC) extracted as a preliminary step to the aldehyde measurements were noted. Yields of GSIC are reported in Table 17. Analysis of variance of the data (Table 18) indicated that the difference in yield with respect to heating rate was significant ($P < 0.05$). Samples heated at the slow rate without respect to end point yielded 1.94 ± 0.36 percent GSIC whereas the fast rate samples yielded 2.3 ± 0.37 percent. A greater decrease in solubility with prolonged heating at the slow rate is indicative of greater denaturation.

End point also had a significant effect on solubility of the heated connective tissue in guanidine hydrochloride. Connective tissue heated to 70°C was significantly ($P < 0.001$) less soluble than that

Table 17. Yield of Guanidine Hydrochloride Soluble Intramuscular Collagen from Connective Tissue Heated in Buffer at Two Rates to Four End Points

Breed	Muscle Number	Yield of GSIC (%)							
		Slow				Fast			
		40°C	50°C	60°C	70°C	40°C	50°C	60°C	70°C
H X C	I	8.27	6.41	5.74	3.30	7.08	7.41	6.83	5.62
	III	1.80	1.36	1.88	0.82	2.00	2.34	2.17	1.20
	VII	1.81	0.54	1.78	0.12	1.52	1.35	1.06	0.44
H X CH	II	1.48	1.01	1.81	0.41	1.49	1.37	2.18	1.11
	IV	1.41	1.09	1.58	0.65	2.56	1.86	1.81	0.46
	V	3.00	2.57	1.91	0.42	2.50	2.80	2.36	0.83
	VI	1.12	0.66	0.73	0.51	1.04	1.20	0.94	0.74
Mean		2.70	1.95	2.20	0.89	2.60	2.62	2.47	1.48
± Standard Error		0.96	0.78	0.61	0.41	0.78	0.83	0.47	0.70

Table 18. Mean Squares and F Values from Analysis of Variance of Percent Yield Guanidine Hydrochloride Soluble Intramuscular Collagen

Source	Degrees of Freedom	Mean Squares	F Values
Rate	1	1.82	6.07*
End Point	3	5.71	19.03***
Linear	1	13.09	43.63***
Quadratic	1	2.18	7.27*
Cubic	1	1.87	6.23*
Rate x End Point	3	0.43	1.43
Breed	1	35.56	1.29
Rate x Breed	1	0.05	1
End Point x Breed	3	0.24	1
Rate x End Point x Breed	3	0.29	1
Animal/Breed[a]	5	27.52	
Treatment x Animal/Breed[b]	35	0.30	

[a]Error term for breed.

[b]Error term for all sources except breed.

*$P < 0.05$; *** $P < 0.001$.

heated to the lower end points (Table 19). The decreased yield of GSIC from 70°C samples might have resulted from solubilization of portions of it during heating or by conversion to a less soluble form via the formation of stronger cross bonding. The formerly postulated change would require breakage of cross linkages, both intra- and intermolecular to form the more soluble α and β components or breakage of peptide linkages to form shorter sections of cross linked peptides.

Since there appear to be factors other than the solubilization of collagen involved in the differences in tenderness of meat heated at two rates, other factors need to be studied. These include the characteristics of the insoluble connective tissue residues as suggested by Pfeiffer et al. (1972) and the effects of heat on the myofibrillar proteins.

Table 19. Mean Percent Guanidine Hydrochloride Soluble Intramuscular Collagen Extracted from Connective Tissue Heated in Buffer to Four End Points

End Point (°C)	Mean GSIC Extracted[a] (%)
40	2.65[b] ± 0.59
50	2.28[b] ± 0.56
60	2.34[b] ± 0.47
70	1.19[c] ± 0.40

[a]Mean and standard error of seven replications.

[b,c]Means with like superscripts do not differ ($P < 0.01$).

CHAPTER V

SUMMARY

Changes in beef semitendinosus cores and isolated semitendinosus intramuscular connective tissue during heating at rates comparable to oven roasting at 93 and 149°C to four end points, 40, 50, 60 and 70°C were investigated. Parameters studied included tenderness of the cores as measured by Warner Bratzler shear, percent connective tissue solubilized in intact muscle tissue and from isolated connective tissue, proteolytic enzyme activity in the cores and drip lost during heating and a number of characteristics of the components of the isolated connective tissue-buffer system.

Tenderness increased ($P < 0.001$) as the internal temperature of the cores increased. Cores heated at the slow rate were more tender ($P < 0.05$) than those heated at the fast rate. The effects of heating rate and end point temperature on tenderness were independent of each other. Cores from Hereford X Charolais-Hereford muscles were more tender ($P < 0.05$) than cores from Hereford X Charolais muscles.

Both heating rate and end point temperature affected the solubilization of connective tissue during heating of the cores. More ($P < 0.01$) connective tissue was solubilized in the slowly heated cores. As the internal temperature increased the percent of connective tissue solubilized increased ($P < 0.001$). The effects of heating rate and end point were dependent on each other. A significant coefficient of correlation ($r = -0.704$; $P < 0.01$) was found between shear values and percent solubilized connective tissue.

General proteolytic activity was exhibited in both water soluble extracts of the cores heated at two rates to four end points and the drip lost during heating. Rate of heating did not significantly affect the loss of activity in the cores, whereas drip from the slowly heated cores exhibited greater ($P < 0.05$) proteolytic activity than the drip from the cores heated at the faster rate. A significant decrease ($P < 0.01$) in activity of cores occurred in the period between 60 and 70°C. In the drip a significant linear decrease ($P < 0.05$) in activity occurred with heating from 40 to 70°C.

End point and rate of heating significantly affected solubilization of connective tissue heated in a buffer system. Solubilization was greater ($P < 0.001$) in the slowly heated samples than in the fast samples. Solubilization increased ($P < 0.001$) as end point temperature increased. Slow 70°C samples contained more labile collagen than fast 70°C samples.

Rate of heating did not significantly affect the length of peptides released during heating. The influence of end point temperature was significant. Peptides in samples heated to 70°C were longer ($P < 0.05$) than those heated to 60°C and below.

As connective tissue was heated in buffer it became less soluble in guanidine hydrochloride. Slowly heated samples were less ($P < 0.05$) soluble than those heated at the fast rate. Samples heated to 70°C were less ($P < 0.001$) soluble in guanidine hydrochloride than samples heated to the lower end points. These results suggest that increased denaturation at the slow rate and higher end point temperatures

resulted in decreased solubility in guanidine hydrochloride.

From the results of this study it appears that connective tissue solubilization is not the only factor that is related to the increased tenderization of meat promoted by heating at slow rates. General proteolytic enzymes may play a role in tenderization during heating. Further characterization of the insoluble connective tissue residues remaining after heating might help to explain the role of connective tissue and changes in it in tenderization of meat. A comparison of the effects of different rates of heating on the myofibrillar component of meat might also help to explain the changes in tenderness that occur with heating.

LIST OF REFERENCES

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APPENDIX

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HYDROXYPROLINE DETERMINATION[a]

Method I--For samples containing at least 2 percent hydroxyproline.

1. To the samples prepared as described previously add 1 ml of Chloramine T, shake and allow to sit for 10 minutes at room temperature.

2. Add 1 ml of 3.15 M perchloric acid, shake and allow to sit for 5 minutes.

3. Add 1 ml of a 20 g/100 ml solution of p-dimethylaminobenzaldehyde in ethylene glycol monomethyl ether and shake until well mixed.

4. Cover tubes and place in a 60°C water bath for 20 minutes.

5. Cool for 5 minutes in tap water.

6. Determine absorbance spectrophotometrically at 557 nm.

Method II--For samples containing less than 2 percent hydroxyproline.

1-5. As in Method I.

6. Add 10 ml of benzene, stopper tube and shake vigorously.

7. After the layers separate, remove the benzene layer with an aspirator.

8. Repeat the extraction with an additional 10 ml benzene.

9. Centrifuge to separate the layers.

10. Carefully introduce a pipet into the water layer and withdraw 3.5 ml and place in a cuvette.

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[a]Woessner, J. F., Jr. 1961. The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. Arch. Biochem. Biophys. 93: 440.

11. Read absorbance at 557 nm. (Steps 6-11 should be completed within 10 minutes.)

12. After the reading, add 0.2 ml of 30 percent H_2O_2 to the cuvette and mix well.

13. Read the absorbance exactly 5 minutes after the peroxide is added.

Standard Curve and Calculations

Prepare a set of standards containing 0, 2.5 and 5 µg hydroxyproline. Treat using the same method as required for the samples.

Values for Method I can be determined directly from the standard curve, a plot of absorbance vs. µg hydroxyproline.

For Method II adjust absorbance values of the standards prior to establishing the standard curve by subtracting the second reading from the first.

Adjust the absorbance values of the samples according to the following equation:

$$(A - B) - 0.12\ (B - C)$$

A = absorbancy after benzene extraction;

B = absorbancy after peroxide treatment;

C = absorbance of water blank after peroxide treatment;

0.12 = empirical factor to correct for fading.

Calculate collagent content from hydroxyproline values as follows:

$$\frac{\text{mg collagen}}{\text{ml or g sample}} = \frac{\text{µg hydroxyproline} \times \text{hydrolysate volume (ml)} \times 10^{-3}}{\text{aliquot volume (ml)} \times \text{sample weight or volume (g or ml)} \times .13^{b}}$$

[b]Conversion factor. Collagen is 13 percent hydroxyproline.

Table 20. Percent Nonfat-Dry Weight of Raw Semitendinosus Muscle and Semitendinosus Cores Heated at Two Rates to Four End Points

Breed	Muscle Number	Percent Nonfat-Dry Weight								
			Slow				Fast			
		Raw	40°C	50°C	60°C	70°C	40°C	50°C	60°C	70°C
H X C	I	23.24	27.07	27.60	29.08	35.02	25.52	27.12	29.10	33.68
	III	23.65	24.04	28.00	28.94	34.18	25.08	27.78	26.30	31.83
	VII	21.97	25.34	26.86	27.98	32.36	24.55	25.76	30.60	34.16
H X CH	II	22.44	24.75	26.33	29.14	35.10	24.41	25.84	26.16	30.18
	IV	23.68	25.38	28.55	28.10	34.63	25.01	27.16	27.40	31.00
	V	23.26	25.68	26.96	28.77	35.20	25.46	26.85	28.84	32.04
	VI	23.82	26.91	26.38	28.78	32.78	25.69	25.90	25.99	32.25
Mean		23.15	25.60	27.24	28.68	34.18	25.10	26.63	27.77	32.16
± Standard Error		0.26	0.41	0.32	0.17	0.44	0.18	0.30	0.67	0.53

Table 21. pH of Raw Semitendinosus Muscle and Semitendinosus Cores Heated at Two Rates to Four End Points

Breed	Muscle Number	pH								
			Slow				Fast			
		Raw	40°C	50°C	60°C	70°C	40°C	50°C	60°C	70°C
H X C	I	5.63	5.55	5.68	5.84	5.84	5.62	5.77	5.85	5.85
	III	5.74	5.84	5.73	5.80	5.86	5.66	5.68	5.68	5.80
	VII	5.66	5.61	5.70	5.71	5.84	5.62	5.71	5.74	5.82
H X CH	II	5.57	5.49	5.76	5.68	5.87	5.62	5.77	5.84	5.84
	IV	5.62	5.53	5.64	5.74	5.85	5.68	5.65	5.72	5.80
	V	5.54	5.59	5.63	5.78	5.81	5.56	5.67	5.76	5.75
	VI	5.70	5.52	5.66	5.87	5.84	5.55	5.65	5.72	5.78
Mean		5.64	5.59	5.69	5.77	5.84	5.62	5.70	5.76	5.81
± Standard Error		0.03	0.04	0.02	0.02	0.01	0.02	0.02	0.02	0.01

Table 22. Aldehyde Content of Guanidine Hydrochloride Soluble Intramuscular Collagen Extracted from Connective Tissue Heated in Buffer at Two Rates to Four End Points

Breed	Muscle Number	Aldehyde Content[a] Slow 40°C	Slow 50°C	Slow 60°C	Slow 70°C	Fast 40°C	Fast 50°C	Fast 60°C	Fast 70°C
H X C	I	8.79	5.95	10.42	9.99	8.78	7.83	7.50	5.73
	III	0.58	8.88	1.32	3.94	0.00	3.57	1.34	3.44
	VII	16.27	11.00	4.16	21.26	1.04	4.20	4.16	8.52
H X CH	II	11.14	18.35	9.16	12.73	14.56	10.36	11.81	5.88
	IV	12.25	1.76	0.24	9.11	1.39	10.45	3.56	7.63
	V	20.34	27.41	3.27	27.03	25.98	18.17	11.14	11.78
	VI	1.31	6.93	7.57	5.39	7.78	2.68	0.43	4.65
Mean		10.10	11.47	5.16	12.78	8.50	8.18	5.70	6.80
± Standard Error		2.75	3.29	1.49	3.20	3.52	2.05	1.72	1.05

[a] $\frac{\mu\text{moles aldehyde}}{\mu\text{mole GSIC}}$.

VITA

Marjorie Porter Penfield was born in Mt. Pleasant, Pennsylvania on May 28, 1942. She attended elementary schools in Connellsville and Pittsburgh, Pennsylvania. In 1960, she graduated from North Allegheny Junior-Senior High School, Pittsburgh, Pennsylvania.

She attended the Pennsylvania State University from September 1960 through July 1966, and received the Bachelor of Science and Master of Science degrees in Foods and Nutrition. While in graduate school at Penn State she held a graduate assistantship. From August 1966 through August 1971, the author was employed as Extension Specialist, Foods and Nutrition and Instructor at Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

In September 1971 she began work as a graduate research assistant at The University of Tennessee, Knoxville. In December 1973 requirements for the Doctor of Philosophy with a major in Food Science were completed. She is a member of the American Home Economics Association, the American Dietetics Association, the Society for Nutrition Education, the Institute of Food Technologists, Phi Upsilon Omicron, Omicron Nu, Sigma Delta Epsilon and Phi Kappa Phi.

The author is the wife of Rogers C. Penfield, Jr. and is the daughter of Mrs. Harry Porter of Export, Pennsylvania and the late Harry P. Porter.