Solubility Characteristics of Residual Collagen as Related to Tenderness of Beef Semimembranosus Roasted at 200° and at 300°F

Cheryl Ann Whetstone

University of Tennessee, Knoxville

Recommended Citation
To the Graduate Council:

I am submitting herewith a thesis written by Cheryl Ann Whetstone entitled "Solubility Characteristics of Residual Collagen as Related to Tenderness of Beef Semimembranosus Roasted at 200° and at 300°F."

I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

Bernadine Meyer, Major Professor

We have read this thesis and recommend its acceptance:

ARRAY(0x7f6ffb909df0)

Accepted for the Council:

Dixie L. Thompson

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)
To the Graduate Council:

I am submitting herewith a thesis written by Cheryl Ann Whetstone entitled "Solubility Characteristics of Residual Collagen as Related to Tenderness of Beef Semimembranosus Roasted at 200° and at 300°F." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science.

[Signature]
Major Professor

We have read this thesis and recommend its acceptance:

[Signature]

[Signature]

Accepted for the Council:

[Signature]
Vice Chancellor for Graduate Studies and Research
SOLUBILITY CHARACTERISTICS OF RESIDUAL COLLAGEN AS RELATED TO TENDERNESS OF BEEF SEMIMEMBRANOSUS ROASTED AT 200° AND AT 300°F

A Thesis
Presented to
the Graduate Council of
The University of Tennessee

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

by
Cheryl Ann Whetstone
August 1969
ACKNOWLEDGMENTS

Deep appreciation is expressed to Dr. Bernadine Meyer for her patient guidance and encouragement throughout the planning, conducting, and reporting of this study. The author also wishes to thank Dr. Grayce E. Goertz and Dr. John T. Smith for their assistance and interest in editing this manuscript. The author is indebted to Dr. William Backus for procuring the meat and for his assistance with sampling of the roasts, to the sensory panel for their participation, to Mrs. Robert Bayne for her assistance with the chemical analyses, to Mrs. Charles Haga for her cooperation in cooking the roasts, and to Miss Judy Simmons and Miss Nancy Kennedy for checking data.

Appreciation is expressed to the American National CowBelles Association and the College of Home Economics for financial assistance, and to the author's parents for their encouragement and support which made possible this advanced degree.

C. A. W.

ii
ABSTRACT

The effects of two heat treatments on cooking time, total cooking losses, energy consumption, meat tenderness, and collagen solubility in 0.1 N NaOH and Ringer's solution were studied using five pairs of top round roasts obtained from three Hereford steers. One roast from each pair was cooked at 200°F and the other at 300°F to an end point of 158°F in the center of the semimembranosus muscle.

Semimembranosus muscles roasted at 200°F required longer cooking times per pound, had higher total cooking losses, consumed more energy, and were more tender than pair mates roasted at 300°F. All correlations between collagen solubility measurements and tenderness measures were nonsignificant. No explanation for the increased tenderness of meat obtained with the long slow heating was revealed by studying the relationship of meat tenderness and collagen solubility in 0.1 N NaOH or Ringer's solution.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>CHAPTER</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>II. REVIEW OF LITERATURE</td>
<td>5</td>
</tr>
<tr>
<td>Relation of Time and Temperature of Heating to Tenderness of Various Beef Cuts</td>
<td>5</td>
</tr>
<tr>
<td>Relation of Alkali Insoluble Protein to Meat Tenderness</td>
<td>8</td>
</tr>
<tr>
<td>Collagen Macromolecule Conformation and Relation of Cross-Bonding to Animal Age</td>
<td>11</td>
</tr>
<tr>
<td>III. PROCEDURE</td>
<td>18</td>
</tr>
<tr>
<td>Source of Meat</td>
<td>18</td>
</tr>
<tr>
<td>Plan of Study</td>
<td>19</td>
</tr>
<tr>
<td>Cooking Procedure</td>
<td>19</td>
</tr>
<tr>
<td>Methods of Evaluation</td>
<td>20</td>
</tr>
<tr>
<td>Physical evaluations</td>
<td>20</td>
</tr>
<tr>
<td>Cooked sample</td>
<td>20</td>
</tr>
<tr>
<td>Raw sample</td>
<td>21</td>
</tr>
<tr>
<td>Chemical evaluations</td>
<td>22</td>
</tr>
<tr>
<td>Sampling</td>
<td>22</td>
</tr>
<tr>
<td>Nonfat dry weight determination</td>
<td>22</td>
</tr>
<tr>
<td>Extraction for collagen determination</td>
<td>22</td>
</tr>
<tr>
<td>Hydrolysis</td>
<td>24</td>
</tr>
</tbody>
</table>
CHAPTER

Hydroxyproline analysis ........................................ 25
Statistical analyses ............................................... 26

IV. RESULTS AND DISCUSSION ................................. 27

Effect of Oven Temperature on Cooking Time, Cooking
Loss, and Energy Consumption ............................... 27
Cooking time ..................................................... 27
Cooking loss ...................................................... 27
Energy consumption ............................................ 29

Effect of Oven Temperature on Tenderness ................. 29

Effect of Oven Temperature on the Solubility Character-
istics of Collagen and the Relationship of These
Characteristics to Meat Tenderness ......................... 33
Alkali insoluble collagen ........................................ 33
Ringer insoluble collagen ....................................... 38
Implications ..................................................... 38

V. SUMMARY ...................................................... 42
Scope of the Study .............................................. 42
Principal Findings .............................................. 42
Implications ..................................................... 43

LIST OF REFERENCES .......................................... 44

VITA ............................................................. 49
### LIST OF TABLES

<table>
<thead>
<tr>
<th>TABLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Summary of Cooking Plan</td>
<td>19</td>
</tr>
<tr>
<td>2. Cooking Data for Top Round Roasted at Two Oven Temperatures</td>
<td>28</td>
</tr>
<tr>
<td>3. Percent Nonfat Dry Weights for the Raw and Cooked Tissue</td>
<td>28</td>
</tr>
<tr>
<td>4. Tenderness Measures for Semimembranosus of Top Round Roasted at Two Oven Temperatures</td>
<td>31</td>
</tr>
<tr>
<td>5. Correlation Coefficients of Tenderness Measures for Semimembranosus of Top Round Roasted at Two Oven Temperatures</td>
<td>32</td>
</tr>
<tr>
<td>6. Alkali Insoluble Collagen Content of the Raw and Cooked Tissue and Percent Decrease in Collagen with Heating at Two Oven Temperatures</td>
<td>34</td>
</tr>
<tr>
<td>7. Correlation Coefficients of Alkali Insoluble Collagen Measures and Meat Tenderness</td>
<td>36</td>
</tr>
<tr>
<td>8. Student's t-test Values for Alkali Insoluble and Ringer Insoluble Collagen Content Between the Raw and Cooked Samples</td>
<td>37</td>
</tr>
<tr>
<td>9. Ringer Insoluble Collagen Content of the Raw and Cooked Tissue and Percent Change in Collagen with Heating at Two Oven Temperatures</td>
<td>39</td>
</tr>
<tr>
<td>10. Correlation Coefficients of Ringer Insoluble Collagen Measures and Meat Tenderness</td>
<td>40</td>
</tr>
</tbody>
</table>
"Consumer studies have shown that tenderness is the most important palatability factor in the acceptance of beef" (Weir, 1960). In spite of years of research there is still much to be done in terms of the underlying factors that are operating in the tenderness of meat. Tenderness is a complex problem and involves at least three muscle components, muscle fibers, fat, and connective tissue. Connective tissue is made up of collagen, elastin, reticulin, and the ground substance. Of these constituents, the one present in the largest amount is collagen which is heat labile (Cover et al., 1962).

"During the cooking of meat, two general changes occur: the muscle fibers become tougher, and the connective tissue becomes more tender" (Weir, 1960). Although moist heat methods generally have not been found beneficial for tender cuts, dry heat methods often seem to be suited for both tender and less tender cuts (Griswold, 1962). The National Live Stock and Meat Board currently recommends a temperature from 300° to 325°F for the roasting of beef. Previous studies in the Food Science Department at the University of Tennessee (Fugate, 1967; Bayne, 1968; Smitherman et al., 1969) and other studies (Cover, 1943; Griswold, 1955; Bramblett et al., 1959; Bramblett et al., 1964) have indicated that roasting at a temperature approximately 100° below the currently recommended temperature resulted in more tender beef than roasting at 300° to 325°F.
While the temperature factor appears to be more important for muscle fiber toughening, the tenderizing effect on connective tissue of low oven temperatures seems to be attributable to the slow heat penetration rather than the low temperatures used (Griswold, 1962; Weir, 1960). Hamm (1966) stated that an increase in tenderness upon cooking of meat cannot be the result of changes in the myofibrillar proteins since they are toughened by heat, but rather must be the result of a change in the collagen of connective tissue. Changes in the collagen of connective tissue generally are believed to be initiated at about 140° to 147°F. These changes include a progressive softening and transformation of collagen into gelatin thereby enhancing tenderness (Bendall, 1964; Lawrie, 1966).

Because residual collagen is believed to be affected by the method of cooking (Ritchey et al., 1962), considerable attention has been given to the methods of determining collagen in meat. Most of the studies done on collagen begin with an extractable collagen (Piez, 1966). Certain solutions, such as dilute alkali, alkali buffers, salt solutions, and acid buffers, are each able to extract a small proportion of collagen from collagenous material. "The insolubility of all but a small proportion of mature collagen in any aqueous or organic solvent that does not attack it chemically is generally indicative of a cross-linked system" (Harding, 1965).

Considerable research has been conducted concerning the alkali insoluble collagen content of bovine muscle tissue. Some workers (Husaini et al., 1950; Loyd et al., 1959) have indicated a significant
negative correlation between alkali insoluble collagen content of raw tissue and meat tenderness. Smitherman et al. (1969) found a significant positive correlation between beef tenderness scores and percent decrease in alkali insoluble collagen of the raw tissue upon roasting at 200° and at 300°F to an end point of 158°F. On the other hand, McClain et al. (1965) reported that the quantity of alkali insoluble collagen of beef after cooking in deep fat (275°F) to an internal temperature of 158°F revealed no significant difference among muscles studied or between animals of two tenderness groups. Regardless of the initial collagen content, the muscles tended to reach a relatively constant collagen content upon cooking. From the contrasting results reported concerning the alkali insoluble fraction of collagen, it may be questioned whether the observed increase in tenderness of meat with cooking could be wholly attributable to the alkali insoluble collagen content of the raw or of the cooked tissues.

Bendall (1964) stated that the toughness of meat cannot be rigidly correlated with collagen content since the cross-bonding parameter will not be taken into effect. The collagen molecule consists of a triple chain helix formed by the twisting together of three polyproline chains held together by intra- and inter-helical cross-bonds (Piez, 1966). During heating, the triple helices of the collagen molecule unravel. In this state, the collagen modulus still offers considerable resistance to the stresses imposed during biting and chewing depending on how highly cross-linked the collagen modulus remains (Bendall, 1964). Wilson et al. (1954) suggested that total amounts of collagen are not always adequate to explain different degrees of toughness.
Several studies have been reported relating age of the animal to tenderness scores of cooked beef tissues. Results have shown that while there was no overall increase in collagen content of the meat with increasing age of the animal, there was a decrease in solubility of the muscle collagen in Ringer's solution (Hill, 1966). Such an effect as decreased solubility in Ringer's solution is presumably attributable to the presence of a higher number of cross-links in the macromolecule of collagen of older animals than of younger animals. Increased solubility of collagen in Ringer's solution was related to increased sensations of tenderness when meat of young animals was compared with that of old animals (Herring et al., 1967).

Since the connective tissue within muscle is believed to play an important role in meat tenderness (Cover et al., 1956; Irwin et al., 1959), the present study was designed to provide further evidence concerning the effect of time and temperature of heating of bovine semimembranosus muscle on: (1) tenderness as measured by sensory evaluations and shear values, and (2) solubility characteristics of collagen as it may relate to tenderness.
CHAPTER II

REVIEW OF LITERATURE

I. RELATION OF TIME AND TEMPERATURE OF HEATING TO TENDERNESS OF VARIOUS BEEF CUTS

Less tender cuts of beef traditionally have been braised rather than cooked by dry heat methods. However, the finding that meat can be made tender by slow heat treatment in the absence of added moisture such as roasting has been supported by research done in several colleges and universities (Cover, 1943; Griswold, 1955; Bramblett et al., 1959; Bramblett et al., 1964; Fugate, 1967; Bayne, 1968).

In an early study by Cover (1943) paired cuts of standing-rib and arm-bone chuck roasts were cooked well done, and bottom-round roasts were cooked both rare and well done at oven temperatures of 257°F and 176°F. Well done roasts cooked at 257°F and 176°F were cooked to end points of 176°F and 158°F respectively. End points for the rare roasts were 145°F for the higher oven temperature and 136°F or 138°F for the lower oven temperature. The longissimus dorsi in the rib, the triceps brachii in the arm-bone chuck, and the biceps femoris in the round were evaluated for tenderness by the paired eating method, weighed adjectives, and Warner-Bratzler shear. For all the roasts cooked at 176°F, the time required for the heat to penetrate the roasts was much longer than for the pair mates cooked at 257°F. Roasts in which the rate of heat penetration was slower also were more tender by all the tenderness measurements used.
Griswold (1955) compared top and bottom muscles of beef round roasted at 250°F and at 300°F with a standard braising method. Shear values for the 250°F roasts were significantly lower than for the 300°F roasts whereas the difference between the shear values of the 250°F roasts and the standard braised meat approached significance, the 250°F roasts having lower shear values.

A study was undertaken by Bramblett et al. (1959) to determine the effect on less tender meat of cooking at very low oven temperatures for long periods of time. Five muscle pairs were dissected from the paired rounds used in the study. The muscles used were the adductor, biceps femoris, gracilis, semimembranosus, and semitendinosus. One of each pair of muscles was roasted at 145°F for 30 hours and the other at 155°F for 18 hours. Warner-Bratzler shear values and sensory panel scores indicated that the meat roasted at 145°F was significantly more tender than paired cuts roasted at 155°F.

To study further the qualities of less tender meat as affected by cooking at low oven temperatures for long periods of time (Bramblett et al., 1964), 60 paired muscles of rounds from 12 beef carcasses were cooked to an internal temperature of 149°F at two oven temperatures, 155°F and 200°F. Results obtained from evaluating tenderness by sensory panel scores, number of chews, L.E.E. Kramer shear press values, and Warner-Bratzler shear values indicated that meat cooked at 155°F was more tender than paired cuts cooked at 200°F. These data supported the previous work of Bramblett et al. (1959).
Nielsen et al. (1965) compared roasting of blade and rump cuts at 225° and at 325°F. Tenderness was evaluated subjectively by sensory panel and objectively by a shearing device developed at the Fish and Wildlife Service of the U. S. Department of the Interior. As determined by shearing tests, blade cuts roasted at 225°F were significantly more tender than those roasted at 325°F, but this difference was not reflected in panel scores. Neither shear values nor scores indicated increased tenderness of rump cuts roasted at 225°F over those roasted at 325°F.

Eighty roasts consisting of small and large, bone-in rib and boneless top round cuts from five high-finish and five low-finish Hereford steers were studied by Bayne (1968). One of each pair was roasted at 300°F and the other at 200°F to end points of 158° and 152°F respectively. Both Warner-Bratzler shear values and panel tenderness scores indicated that roasts subjected to the 200°F heat treatment were more tender than those roasted at 300°F with the improvement in tenderness being more pronounced for the rounds than for the rib cuts.

In a similar study Fugate (1967) compared rib roasts cooked at 225° and at 325°F to end points of 140°, 158°, and 170°F. Panel scores indicated that roasts cooked at 225°F to each end point temperature and those at 325°F cooked to 140°F were significantly more tender (P < .01) than roasts cooked at 325°F to end points of 158° or 170°F. Roasts cooked at 225°F to end points of 158° and 170°F sheared significantly lower than roasts cooked by all other heat treatment combinations.

As mentioned in the introduction, tenderness is a complex problem involving the muscle fibers, fat, and connective tissue. In none of the
studies reviewed was any attempt to explain the improved tenderness with the low temperature on the basis of chemical changes in the proteins.

II. RELATION OF ALKALI INSOLUBLE PROTEIN TO MEAT TENDERNESS

When meat is cooked or heated, a fraction of the collagen in connective tissue may be converted to gelatin; the amount not converted is believed to be one important factor in the overall toughness of meat. Consequently, considerable attention has been given to methods of determining collagen in meat.

The partition and analysis of the vaguely defined components of connective tissue are limited by the flaws in, and length of, existing methods. In the past 20 years, investigations relating the hydroxyproline content of the alkali insoluble protein of muscle to the tenderness of meat have appeared in the literature (Husaini et al., 1950; Wierbicki et al., 1954; Loyd et al., 1959). These and other studies were aimed toward collagen estimation based upon the insolubility of collagen in 0.1 N NaOH. Although the alkali insoluble collagen is actually a measure of the collagen solubility in dilute alkali, all too often it has been interpreted as an indication of the amount of collagen. This should be kept in mind in reviewing the literature.

In an early study by Husaini et al. (1950), 20 carcasses of wide variation in grade, age, and sex were sampled by excising one short loin from each carcass. Samples of the raw tissue were ground and blended with distilled water to form a slurry. The alkali insoluble protein.
remaining after the extraction of the slurry with dilute alkali, was hydrolyzed and analyzed for hydroxyproline content. A negative correlation (P < .01) between tenderness scores of the cooked tissue and the alkali insoluble proteins of the raw tissue was obtained.

Loyd et al. (1959) investigated the relationship of hydroxyproline content of the alkali insoluble protein of raw muscle to the tenderness of cooked meat as measured by sensory panel and Warner-Bratzler shear. Two methods of collagen fractionation were presented. In the first method, which required five or six days for completion, each sample was ground three times, blended once, weighed twice, centrifuged five times and autoclaved three times. On the other hand, the second method required only three days. In this method each sample was comminuted once, weighed once, centrifuged twice, and autoclaved once. Both methods of fractionation showed a significant negative correlation between the hydroxyproline content of the alkali insoluble protein of the raw tissues and tenderness of the cooked tissue by the two tenderness measures employed.

Collagen was determined by nitrogen and by hydroxyproline in the alkali insoluble, autoclave soluble fraction of raw steaks, as well as comparable ones cooked by dry heat to an internal temperature of 142°F and on others cooked by moist heat to 212°F and held at this temperature for 25 minutes (Ritchey et al., 1962). The dried and pulverized samples were extracted with alkali according to a modified Lowry et al. (1941) procedure. Collagen nitrogen values measured by hydroxyproline were consistently lower than those measured by micro-Kjeldahl in raw, rare
(142°F), and well done (212°F) steaks. Reduction of collagen was much greater in well done samples than in rare steaks.

McClain et al. (1965) in an effort to clarify the role and specific relationship of collagen to the tenderness of beef muscle, studied the relationship of alkali insoluble collagen to shear value of bovine muscle. Twenty-eight carcasses were selected from 118 yearling steers based upon preliminary shear values, and then grouped according to shear value into tender and less tender groups. Steaks from the longissimus dorsi, semimembranosus, and triceps brachii muscles were cooked in deep fat (275°F) to an internal temperature of 158°F. Warner-Bratzler shear values were obtained on the cooked tissue and used in relating meat tenderness to alkali insoluble collagen content of the raw and cooked samples. No significant difference in the quantity of alkali insoluble collagen was found between tenderness groups for triceps brachii and longissimus dorsi muscles, and only a slight difference (P < .05) was observed in the semimembranosus muscles. Regardless of initial collagen content, muscles tended to reach a relatively constant collagen content upon cooking and therefore no significant difference in collagen content of the cooked meat among muscles studied or between tenderness groups was revealed. Results indicated that absolute quantities of alkali insoluble collagen of cooked muscles were not related to shear values of either cooked or uncooked muscles. However, differences were observed in the percent alkali insoluble collagen converted to gelatin upon cooking, indicating differences in type or characteristics of the muscle collagen.
Similar results were obtained by Smitherman et al. (1969) in semimembranosus muscle roasted at 200° and at 300°F to an end point of 158°F. Alkali insoluble collagen content was determined on the raw and the cooked tissue. Results indicated that the amount of alkali insoluble collagen of the cooked tissue was not related to shear values or tenderness scores of the cooked tissue. While there was a greater decrease in residual collagen from the raw to the cooked tissue in the roasts cooked at 200°F than in pair mates cooked at 300°F, this difference was not significant.

From the contrasting results reported concerning the connective tissue component of meat tenderness, it seems unlikely that the observed increase in tenderness of meat upon cooking or the decrease in tenderness with increased age of the animal can be wholly attributable to the alkali insoluble collagen content of beef tissue. More knowledge is needed concerning the nature of the collagen macromolecule.

III. COLLAGEN MACROMOLECULE CONFORMATION AND RELATION OF CROSS-BONDING TO ANIMAL AGE

It is necessary to know the conformation of the molecular chain as well as the complete amino acid sequence of collagen to understand collagen activity in biological systems. Conformation of the molecular chain is the way in which the chain is folded and the way in which the side chains are positioned with respect to the folded or the coiled backbone. The full amino acid sequence is as yet unknown, but it was realized long ago from the appearance of its x-ray diffraction pattern
that the collagen backbone is based on some type of folding or coiling (Ramachandran et al., 1962; Ramachandran, 1967).

The final breakthrough, which led to the correct structure, came not directly from the x-ray pattern, but from stereochemical considerations. These stereochemical considerations, namely the postulate that every third residue must be glycine and that proline and hydroxyproline residues must naturally fit into the structure, led to a triple-helical structure, with the three helices standing side by side and linked to one another by means of hydrogen bonds nearly perpendicular to the fiber axis (Ramachandran, 1967).

Each chain of collagen is a polyproline helix and the three helices are twisted or coiled into a larger chain helix. There are generally two kinds of chains in each molecule, designated α1 and α2 (Piez, 1968). Synthesis of the subunits of the triple stranded collagen molecule proceeds by the same route as that of the soluble proteins (Fruton, 1963). To produce the functioning unit, the collagen fibril, at least four additional steps following polypeptide synthesis are required. "Some of the prolyl and lysyl residues are hydroxylated; carbohydrate is attached; molecules are aggregated in a specific manner into fibrils; and inter-chain (both intra- and intermolecular) covalent cross-links are produced during and after aggregation" (Piez, 1968). The intramolecular cross-links are links between the individual chains of the triple helical collagen, whereas the intermolecular cross-links are links from one collagen monomer to another (Harding, 1965).
Jackson et al. (1960) reported that there are no clear cut collagen fractions, but a continuous spectrum of molecular aggregates of increasing age and that the maturation of the collagen aggregates was marked by an increase in the strength of cross-linkages and by a change in the solubility characteristic of the collagen. According to Harding (1965) the degree and rate of cross-linking are characteristic of the tissue and may vary with age. These properties presumably bear an important relationship to collagen function.

It is not possible to analyze for cross-link content directly since the chemical nature of the cross-link is not understood, but the degree to which collagen can be extracted from a tissue by cold neutral salt or dilute acid solutions is an approximate measure of covalent cross-linking. Most of the studies done on collagen begin with an extractable collagen (Piez, 1966). "The insolvency of all but a small proportion of mature collagen in any aqueous or organic solvent that does not attack it chemically is generally indicative of a cross-linked system" (Harding, 1965).

Limited research has been conducted on the age-associated changes in muscle composition of meat animals. Hill (1966) in reviewing the changes in the structure of collagen with increasing age of the animal reported Verzar's postulation that, "since collagen does not have a metabolic turnover, molecular movements eventually bring the polypeptide chains nearer to each other, thus assisting the formation of cross-linkages." Hill (1966) placed dried and pulverized samples in a tube with Ringer's solution in a 171°F water bath for 63 minutes. The
resulting residues and supernatant fluids were hydrolyzed and analyzed for hydroxyproline content using a modified Ritchey et al. (1963) procedure. While there was no overall increase in collagen content with age, solubility in Ringer's solution decreased in the sternomandibularis muscles of 42 Holstein Friesian bovines studied. The results of Hill (1966) can be considered as strong presumptive evidence of an increase in the number or strength of the cross-linkages of intramuscular collagen in meat animals as they age. Hill (1966) indicated that it is likely that additional factors which depend on the age of the animal, other than cross-bonding, are involved in determining the degree of solubility of collagen in Ringer's solution.

In a similar study by Herring et al. (1967), the longissimus dorsi and semimembranosus muscles of 15 bovine animals equally divided among A, B, and E maturities (U.S.D.A.) were used. Frozen powdered samples were placed in centrifuge tubes with Ringer's solution. The samples were heated for 70 minutes in a 171°F water bath and stirred occasionally. After centrifugation, the supernatant fluids were decanted and the residues washed with Ringer's solution. Acid hydrolysates of the supernatant and residue were analyzed for hydroxyproline according to a modification of the procedure of Woessner (1961). Collagen solubility in Ringer's solution decreased significantly with each advancing maturity group for both longissimus dorsi and semimembranosus muscles. Collagen solubility also was related to tenderness of both muscles as evaluated by sensory panel and shear measurement.
The findings of Herring et al. (1967) are in agreement with those reported by Goll et al. (1964 a,b,c) who studied the effect of age of the animal on the rate of hydrolysis by collagenase, exposure to increasing temperature, and rate of solubilization of bovine muscle connective tissue. The samples for these three studies were from four age groups: Group I, veal, 40 to 49 days old; Group II, steers, 403 to 495 days old; Group III, cows, four years, eight months to five years, five months old; Group IV, aged cows, 10 years, two months to 10 years, five months old. These studies were conducted to investigate age-associated structural changes in collagenous residues obtained from the loose connective tissue found within bovine biceps femoris. Group I was followed by Groups III, IV, and II from fastest to slowest in rate of digestion by collagenase as indicated by ninhydrin and hydroxyproline analyses. It was postulated that "the larger amounts of lipid associated with the Group II samples acted to shield collagenase-labile bonds and cause a slow rate of hydrolysis" (Goll et al., 1964 a). In discussing the results obtained in the study, they stated that "although change in the rate of enzymatic digestion provides presumptive evidence for the existence of more frequent or stronger cross-linkages in more mature collagen, it does not, by itself, rule out the effect of insoluble substrates of shielding collagenase-susceptible bonds."

Since strengthening or increasing the number of interchain cross-linkages leads to an increase in the thermal shrinkage temperature, this temperature appeared to offer a method for obtaining additional information regarding changes in collagen structure upon maturation and the
relation of these changes to meat tenderness. This was the postulate of the study by Goll et al. (1964 b). The changes in thermal shrinkage were followed by analyzing for the soluble ninhydrin positive material and hydroxyproline released from the collagen fibers during heating at temperatures of 77°, 113°, 131°, 149°, and 158°F. The ninhydrin and hydroxyproline analyses showed marked age-associated differences among the four groups indicating that collagen from more mature animals possessed more frequent or stronger cross-linkages.

To provide additional information on the nature and strength of the cross-linkages existing in the collagen fibers from animals of different ages, a third study of the rate of solubilization of insoluble bovine muscle connective tissue residues in a boiling water bath was undertaken by Goll et al. (1964 c). The release of soluble protein, ninhydrin and soluble hydroxyproline indicated that collagen from the younger animals was solubilized (i.e. converted to gelatin) in a 212°F water bath more rapidly than collagen from the older animals.

The physiocochemical tests presented by Goll et al. (1964 a,b,c) indicated that collagen isolated from the loose connective tissue network within bovine muscle changes in character with maturation of the animal. Mature collagen appeared to contain more extensive or stronger cross-linkages than collagen from younger animals. These studies did not reveal any differences in the total collagen content of the fresh muscle among the four maturity groups. However, some simple tests indicated that tenderness decreased with increasing age of the animal. The results suggested that the cooking process may have had less effect on
the connective tissue in meat cuts from the older animals than from the younger animals, thereby leaving the collagen relatively undegraded with resultant less tenderness of the meat cuts (Goll et al., 1964 c).
CHAPTER III

PROCEDURE

I. SOURCE OF MEAT

Six pairs of top round roasts were obtained from three Hereford steers produced in the University of Tennessee research herd at Franklin, Tennessee. These calves were fed a ration which consisted of corn silage ad libitum, 4.5 lb ground corn and 1.5 lb cottonseed meal per day.

The cattle, ranging in age from 12 to 16 months, were slaughtered March 19, 1969, at East Tennessee Packing Company, Knoxville. The carcasses were brought to the University of Tennessee meat cooler and aged six days at 32 ± 2°F prior to cutting. The average carcass weight was 386 lb.

Two pairs of 4-in thick top round roasts containing the semimembranosus, gracilis, and adductor muscles were removed from each carcass. Sampling was done at the articulation between the pelvis and femur. Pair one, the more anterior of the two pairs, was taken measuring four inches down from the hip. Pair two was removed adjacent to pair one toward the shank end.

Roasts were wrapped in freezer paper, frozen at -20°F and stored at -5°F until the day prior to cooking.
II. PLAN OF STUDY

One roast from each pair was cooked at 200°F and the other at 300°F to an end point of 158°F in the center of the semimembranosus, the test muscle. One pair of roasts was used for preliminary testing and five pairs of roasts were used for the present study. The cooking plan is shown in Table 1.

Table 1. Summary of cooking plan.

<table>
<thead>
<tr>
<th>Animal number</th>
<th>Pair number</th>
<th>Side</th>
<th>Weight (lb)</th>
<th>Oven temperature (°F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>207</td>
<td>1</td>
<td>Left</td>
<td>2.5</td>
<td>300</td>
</tr>
<tr>
<td>207</td>
<td>1</td>
<td>Right</td>
<td>2.8</td>
<td>200</td>
</tr>
<tr>
<td>153</td>
<td>1</td>
<td>Left</td>
<td>2.7</td>
<td>200</td>
</tr>
<tr>
<td>153</td>
<td>1</td>
<td>Right</td>
<td>3.1</td>
<td>300</td>
</tr>
<tr>
<td>153</td>
<td>2</td>
<td>Left</td>
<td>2.8</td>
<td>300</td>
</tr>
<tr>
<td>153</td>
<td>2</td>
<td>Right</td>
<td>2.5</td>
<td>200</td>
</tr>
<tr>
<td>202</td>
<td>1</td>
<td>Left</td>
<td>3.5</td>
<td>300</td>
</tr>
<tr>
<td>202</td>
<td>1</td>
<td>Right</td>
<td>3.3</td>
<td>200</td>
</tr>
<tr>
<td>202</td>
<td>2</td>
<td>Left</td>
<td>2.7</td>
<td>200</td>
</tr>
<tr>
<td>202</td>
<td>2</td>
<td>Right</td>
<td>2.7</td>
<td>300</td>
</tr>
</tbody>
</table>

III. COOKING PROCEDURE

One day prior to cooking, the roasts were defrosted at room temperature (70°F to 72°F) to an internal temperature of 32°F in the approximate center of the test muscle and then refrigerated overnight.
On the day of cooking, the roasts were wiped free of any drip which may have accumulated and weighed to the nearest gram on a Mettler K-7 balance. All roasts were placed fat-side up on racks in tared roasting pans. A thermocouple was inserted into the approximate geometric center of the semimembranosus muscle of the round cuts. The internal temperature at the center of the semimembranosus muscle of the roasts as well as the oven temperature was measured throughout the roasting period by copper-constantan thermocouples and recorded automatically at 4-min intervals by a multipoint temperature recorder.

Roasts were cooked in two household electric ranges in which the temperature fluctuation was \( \pm 10^\circ F \). A watt-hour meter was connected to the power line of each range and power consumption not including the preheating periods for the ranges was measured. Cooking periods in preheated ovens were begun so that roasts cooked at both oven temperatures would be ready for sensory evaluation at approximately the same time. When the internal temperature of the test muscle reached 158°F, cooking was terminated and the roasts were cooled at room temperature for 30 minutes. Weights were taken for the determination of evaporative, drip, and total cooking losses.

IV. METHODS OF EVALUATION

Physical Evaluations

Cooked sample. After cooking data were obtained, the semimembranosus muscle was separated from the adductor and gracilis muscles. The outer slice from the anterior side of the muscle was discarded and
three adjacent slices approximately 0.6 cm thick were removed from the roast to provide six samples for sensory evaluations. Two 1-in discs were taken from the center of each slice for chew count evaluations and samples for scoring were obtained from each side of the slice.

An experienced panel of six evaluated the roasts for tenderness by scoring and by chew count. Tenderness was evaluated on an 11-point descriptive scale with a score of 10 representing maximum quality. Samples for each judge were obtained from the same location of each roast. The panel scored coded paired samples roasted at 200° and at 300°F from one muscle pair each scoring period.

Tenderness was evaluated objectively by a Warner-Bratzler shear. Shear force values of the cooked samples were obtained on four 1-in cores approximately 2-in long, from the posterior side of the roasts. Cores were cooled at room temperature for 30 minutes, sheared three times on a Warner-Bratzler shear, and average shear values calculated for each roast.

**Raw sample.** One day prior to cooking, a 1-in thick slice was removed from the posterior side of the frozen roasts for evaluation of the raw tissue. After thawing the raw slice to 32°F in the center of the semimembranosus muscle, four 1-in cores were taken beginning with the end nearer the adductor. Cores were sheared at 50°F and shear values averaged for each roast.
Chemical Evaluations

Sampling. The semimembranosus muscle of the raw and cooked tissue was trimmed of epimysial connective tissue, diced, and frozen with the cores which had been sheared. The meat samples were ground in the frozen state with a Hobart N-50 electric meat grinder four times through fine mesh and mixed well.

Approximately 2-g samples for collagen determination were weighed by difference to the nearest milligram on the day of grinding and frozen for lyophilization. At the same time, samples were obtained for nonfat dry weight determination.

Nonfat dry weight determination. Approximately 5 g of the ground raw and cooked tissue samples were weighed by difference to the nearest milligram into tared Whatman 22 x 80 mm single thickness extraction shells. After drying for four hours in a warm oven with the door open, the samples were placed in a vacuum oven and dried overnight (15.5 hours) at 149°F. The moisture content of the meat samples was calculated from the weight of the samples after cooling approximately 1.5 hours in a dessicator.

The dried samples were then extracted with petroleum ether (boiling range 86° to 140°F) on a Goldfisch apparatus for a total of 10 hours. After extracting, drying and cooling, the samples were reweighed to obtain the nonfat dry weight of the samples.

Extraction for collagen determination. Collagen was determined on the alkali insoluble residue and the Ringer insoluble residue of the
raw and cooked semimembranosus muscles. The alkali extraction procedure used was a modification of the procedure of Ritchey et al. (1963), and the Ringer extraction procedure used was a modification of the procedure of Goll et al. (1963).

Solutions:

1. Sodium Hydroxide (0.1 N): 4.2 g of NaOH were diluted to 1000 ml with distilled water.

2. Ringer's Solution: The solution contained per 1000 ml:
   - 6.500 g NaCl
   - 0.140 g KCl
   - 0.240 g CaCl₂·2H₂O
   - 0.500 g NaHCO₃

Iodoacetate, 0.186 g, was added prior to making to volume to prevent microbial spoilage and collagenase activity in the meat during the extractions.

Procedure:

After lyophilization, duplicate samples for the raw and cooked meat were pulverized with mortar and pestle and transferred to 50-ml nalgene centrifuge tubes with 35 ml of 0.1 N NaOH which was warmed to 95° to 98°F. Samples were stirred with glass stirring rods, tubes capped with parafilm (secured with rubber bands) and placed in a water bath shaker maintaining a temperature range of 95° to 98°F and with the speed control positioned between medium and high. After extracting for four hours, samples were chilled to about 38°F by storing in a freezer 45
minutes. The chilled samples were centrifuged at 2100 x gravity for 45 minutes. The centrifuge was kept in a cold room averaging a temperature of 42°F. Supernatant fluids were decanted through Gooch crucibles lined with glass wool (thick mat in bottom). To the residues left in the centrifuge tubes, 30 ml of 0.1 N NaOH were added and the tubes again were capped with parafilm and placed in the water bath adjusted as described above and extracted for approximately 18 hours. After the second extraction, the supernatants were decanted through, and the residues washed into, the Gooch crucibles used after the first extraction. Each residue was washed six times with 25 ml of distilled water at 95°F. The glass wool was dried with suction and with the residue was carefully transferred to 50-ml screw cap test tubes. The extracted samples were frozen in the test tubes until ready for hydrolysis. Samples for the Ringer extraction were treated in the same manner except Ringer's solution containing iodoacetate was used in lieu of the 0.1 N NaOH for the extracting medium.

**Hydrolysis.** Ten ml of 20.2% HCl were added to each tube containing the extraction residues and the samples were hydrolyzed in an autoclave at 15 psi for nine hours. Hydrolysates were refrigerated overnight and then decolorized with activated charcoal by shaking mechanically for one hour. The contents were filtered through folded filter paper and made to proper dilution. The hydrolysates of the alkali extraction were diluted to 50 ml while the hydrolysates of the Ringer extraction were diluted to 100 ml. The diluted hydrolysates were transferred to 4-oz
bottles. Samples were held in freezer storage until used for hydroxyproline determination.

Hydroxyproline analysis. The method of analysis employed was a modification of the procedure of Martin et al. (1953) which was a modification of the basic Neuman et al. (1950) method.

Reagents:

1. Standard Hydroxyproline Stock Solution: 0.2500 g of Hydroxy L proline (Nutritional Biochemicals Corporation) was dissolved in sufficient water to make 1000 ml. Operational standard solutions were prepared as follows: 10 ml of the stock standard plus 10 ml of 20.2% HCl were diluted to 100 ml with distilled water.

2. Copper Sulfate Solution (0.25%): 2.5 g of CuSO\(_4\)·5H\(_2\)O were dissolved in sufficient water to make 1000 ml.

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

4. Hydrogen Peroxide Solution (6%): 20 ml of 30% H\(_2\)O\(_2\) were diluted to 100 ml with distilled water. Made fresh for each determination.

5. Ferrous Sulfate Solution (0.05 M): 1.4 g of FeSO\(_4\)·7H\(_2\)O and 0.5 ml concentrated H\(_2\)SO\(_4\) were dissolved in sufficient distilled water to make 100 ml. Made fresh for each determination.

6. Ehrlich's Reagent (5%): 25 g of para-dimethyl-aminobenzaldehyde were dissolved in sufficient 1-propanol to make 500 ml.

7. Sulfuric Acid (4.0 N): 220 ml of 97% H\(_2\)SO\(_4\) (sp gr 1.84) were diluted to 2000 ml with distilled water.
Procedure:

Aliquots of the hydrolysates and the operational standard containing from 2.5 to 20.0 µg of hydroxyproline were pipetted in duplicate into a series of 18 x 150 mm Pyrex test tubes. After the volume in each tube was adjusted to 1 ml with distilled water, 2 ml of a 1:1 (v:v) mixture of CuSO₄ and NaOH solutions were added. (These two reagents were mixed immediately prior to use.) One ml of the peroxide solution was then added and the samples were shaken for five seconds. After the last tube had received H₂O₂, the samples were shaken on a mechanical shaker for 15 minutes. One-tenth ml of the FeSO₄ was added to each sample to remove any excess peroxide and the samples were again shaken for 10 minutes with a mechanical shaker. Next, 5 ml of a 2:3 (v:v) mixture of Ehrlich's reagent and 4.0 N H₂SO₄ were added. The tubes were capped with marbles and held in a water bath for 20 minutes for color development, then cooled in tap water for five minutes. Optical density of the samples was then determined on a Spectronic 20 at 560 µm.

Statistical Analyses

Student's t-test for significant difference between means of the samples roasted at 200°F and at 300°F, between means of the corresponding raw samples, and between means of the raw and cooked samples for each roasting temperature were determined. Correlation coefficients were determined for Ringer insoluble collagen with tenderness scores, chew count, and shear values; alkali insoluble collagen with tenderness scores, chew count, and shear values; chew count with shear values; chew count with tenderness scores; and shear values with tenderness scores.
CHAPTER IV

RESULTS AND DISCUSSION

I. EFFECT OF OVEN TEMPERATURE ON COOKING TIME,
COOKING LOSS, AND ENERGY CONSUMPTION

Cooking Time

The cooking time per pound was significantly longer (P < .001, Table 2) for the roasts cooked at 200°F than for pairs cooked at 300°F. As shown in Table 2, a mean of 213 minutes per pound was required for roasts cooked at 200°F whereas, a mean of 33 minutes per pound was required for roasts cooked at 300°F. The association of long cooking times with low oven temperatures was consistent with results obtained by other investigators (Cover, 1943; Bramblett et al., 1959; Bramblett et al., 1964; Griswold, 1955; Bayne, 1968; Smitherman et al., 1969).

Cooking Loss

Total cooking losses were higher (P < .001) for the 200°F roasts than the 300°F roasts as presented in Table 2. The major proportion of the total cooking loss at both oven temperatures was attributable to evaporation. The average evaporative loss expressed as a percentage of the total cooking loss was 92% for the 200°F roasts and 60% for the 300°F roasts. The percent nonfat dry weights of the roasts cooked at 200°F were significantly higher (P < .001, Table 3) than for roasts cooked at 300°F. No significant difference was found between the percent nonfat dry weights of the raw samples.
Table 2. Cooking data for top round roasted at two oven temperatures.1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Oven temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>200°F</td>
</tr>
<tr>
<td>Cooking time (min/lb)</td>
<td>213 ± 30</td>
</tr>
<tr>
<td>Total cooking losses (%)</td>
<td>34.9 ± 1.5</td>
</tr>
<tr>
<td>Energy consumption (Kwatt-hr/lb)</td>
<td>0.66 ± 0.16</td>
</tr>
</tbody>
</table>

1Results expressed as mean ± standard deviation.

***P < .001.

**P < .01.

Table 3. Percent nonfat dry weights for the raw and cooked tissue.

<table>
<thead>
<tr>
<th>Animal number</th>
<th>Pair number</th>
<th>Raw 200°F</th>
<th>Raw 300°F</th>
<th>Cooked 200°F</th>
<th>Cooked 300°F</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>207</td>
<td>1</td>
<td>24.6</td>
<td>24.5</td>
<td>34.1</td>
<td>32.0</td>
<td>0.1</td>
</tr>
<tr>
<td>153</td>
<td>2</td>
<td>25.2</td>
<td>24.1</td>
<td>35.5</td>
<td>33.4</td>
<td>2.5</td>
</tr>
<tr>
<td>153</td>
<td>1</td>
<td>24.0</td>
<td>24.2</td>
<td>34.4</td>
<td>31.4</td>
<td></td>
</tr>
<tr>
<td>202</td>
<td>2</td>
<td>25.0</td>
<td>24.9</td>
<td>34.8</td>
<td>32.2</td>
<td></td>
</tr>
<tr>
<td>202</td>
<td>1</td>
<td>23.9</td>
<td>24.0</td>
<td>33.9</td>
<td>31.0</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>24.5</td>
<td>24.4</td>
<td>34.5</td>
<td>32.0</td>
<td></td>
</tr>
<tr>
<td>Difference</td>
<td></td>
<td>0.1</td>
<td></td>
<td>2.5</td>
<td></td>
<td>***</td>
</tr>
</tbody>
</table>

1P > .05.

***P < .001.
Smitherman et al. (1969) reported that total cooking losses for beef round roasted at 200° and at 300°F were 28.3% and 25.7% respectively. The difference was significant. Bayne (1968) found a trend toward higher cooking losses for boneless round cuts cooked at 200° than at 300°F. Other workers (Griswold, 1955; Simmers, 1965) reported that losses for top round cuts were higher for roasts cooked at the low oven temperature than for roasts cooked at the recommended temperature of 300° to 325°F.

Energy Consumption

Energy consumption was affected by oven temperature (P < .01, Table 2). The average energy consumed on a per pound basis was 0.66 kilowatt-hours for the 200°F roasts and 0.22 kilowatt-hours for the 300°F roasts.

Other studies have shown that cooking at low oven temperatures required more energy. Simmers (1965) reported that semimembranosus muscles roasted at 200°F required approximately 50% more watt-hours per pound than paired cuts roasted at 300°F. Nielsen et al. (1965) found that the long slow cooking of blade roasts at 225°F required an average of 1.3 more kilowatt-hours of electricity than did the shorter cooking at 325°F.

II. EFFECT OF OVEN TEMPERATURE ON TENDERNESS

Shear values for the raw meat indicated no differences in the tenderness of the semimembranosus muscle prior to cooking. Treatment means for tenderness scores, chew count, and shear values of the cooked
tissue are presented in Table 4. By both objective and subjective measures, the roasts cooked at 200°F were assessed to be significantly more tender than pair mates cooked at 300°F. The mean panel score for tenderness of the roasts was 8.5 for those cooked at 200°F and 6.3 for the ones cooked at 300°F. The average chew-count value for the 200°F roasts was 26.4 and for the 300°F roasts, 36.2. The trend toward higher tenderness scores and lower chew-count values for the 200°F roasts than for the 300°F roasts was consistent.

A mean of 11.5 lb of force was required to shear cores from the roasts cooked at 200°F whereas, 19.0 lb of force were required to shear cores from the 300°F roasts indicating the roasts cooked at 200°F were significantly more tender (P < .01, Table 4) than the roasts cooked at 300°F.

Correlation coefficients for the three measures of tenderness are presented in Table 5. Panel scores for tenderness were negatively correlated with shear values and chew count. Shear values were positively correlated with chew count.

The findings of the present study indicated that roasting at 200°F had a greater tenderizing effect on semimembranosus muscle than roasting at 300°F. Previous investigators have reported similar results. In an early study by Cover (1943), standing-rib, armbone-bone chuck, and bottom-round roasts cooked at 176°F were evaluated by sensory panel and Warner-Bratzler shear to be more tender than pair mates cooked at 257°F. Griswold (1955) found top and bottom muscles of the round roasted at 250°F were significantly more tender than muscles roasted at 300°F as
Table 4. Tenderness measures for semimembranosus of top round roasted at two oven temperatures.

<table>
<thead>
<tr>
<th>Animal number</th>
<th>Pair number</th>
<th>Tenderness score&lt;sup&gt;1&lt;/sup&gt; Oven temperature</th>
<th>Chew count Oven temperature</th>
<th>Shear value Oven temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>200°F</td>
<td>300°F</td>
<td>200°F</td>
</tr>
<tr>
<td>207</td>
<td>1</td>
<td>8.7</td>
<td>6.2</td>
<td>26.3</td>
</tr>
<tr>
<td>153</td>
<td>2</td>
<td>8.2</td>
<td>5.7</td>
<td>26.2</td>
</tr>
<tr>
<td>153</td>
<td>1</td>
<td>9.3</td>
<td>7.2</td>
<td>22.7</td>
</tr>
<tr>
<td>202</td>
<td>2</td>
<td>7.8</td>
<td>6.0</td>
<td>29.0</td>
</tr>
<tr>
<td>202</td>
<td>1</td>
<td>8.7</td>
<td>6.3</td>
<td>28.0</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>8.5</td>
<td>6.3</td>
<td>26.4</td>
</tr>
<tr>
<td>Difference</td>
<td></td>
<td>2.2***</td>
<td></td>
<td>9.8***</td>
</tr>
</tbody>
</table>

<sup>1</sup> Maximum score, 10.

***P < .001.

**P < .01.
Table 5. Correlation coefficients of tenderness measures for semimembranosus of top round roasted at two oven temperatures.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>r values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tenderness score vs</td>
<td></td>
</tr>
<tr>
<td>Shear value</td>
<td>-0.952**</td>
</tr>
<tr>
<td>Chew count</td>
<td>-0.978**</td>
</tr>
<tr>
<td>Shear value vs Chew count</td>
<td>0.942**</td>
</tr>
</tbody>
</table>

**p < .01.
evaluated by shear values. Bayne (1968) reported round and rib cuts roasted at 200°F were evaluated more tender by sensory panel and shear values than pair mates roasted at 300°F. Other studies have indicated similar results (Bramblett et al., 1959; Bramblett et al., 1964; Nielsen et al., 1965; Fugate, 1967).

III. EFFECT OF OVEN TEMPERATURE ON THE SOLUBILITY CHARACTERISTICS OF COLLAGEN AND THE RELATIONSHIP OF THESE CHARACTERISTICS TO MEAT TENDERNESS

The primary objective of the present study was to investigate the effect of time and temperature of heating bovine semimembranosus muscle on the solubility characteristics of collagen in dilute alkali and Ringer's solution and to relate these factors to differences in tenderness.

Alkali Insoluble Collagen

The amount of collagen on a nonfat dry weight basis remaining after extraction of the raw and cooked tissue with dilute alkali (0.1 N NaOH) is presented in Table 6. No significant difference was found between the alkali insoluble collagen of the raw tissues or between the residual collagen content of the roasts cooked at 200° and at 300°F. No significant difference was found between percent decrease of the alkali insoluble collagen from the raw to the cooked tissue for the two oven temperatures. These results are in agreement with results reported by Smitherman et al. (1969). They found no significant difference between alkali insoluble collagen content of the tissue cooked at 200° or at
Table 6. Alkali insoluble collagen content of the raw and cooked tissue and percent decrease in collagen with heating at two oven temperatures.

<table>
<thead>
<tr>
<th>Animal number</th>
<th>Pair number</th>
<th>Alkali insoluble collagen</th>
<th>Percent decrease with heating</th>
<th>Oven temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mg collagen/g meat¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Raw 200°F 300°F</td>
<td>Cooked 200°F 300°F</td>
<td></td>
</tr>
<tr>
<td>207</td>
<td>1</td>
<td>22.65 19.83</td>
<td>5.01 7.04</td>
<td>77.9 64.5</td>
</tr>
<tr>
<td>153</td>
<td>2</td>
<td>17.87 18.67</td>
<td>5.55 4.77</td>
<td>68.9 74.4</td>
</tr>
<tr>
<td>153</td>
<td>1</td>
<td>24.45 24.25</td>
<td>5.40 6.90</td>
<td>77.9 71.6</td>
</tr>
<tr>
<td>202</td>
<td>2</td>
<td>13.03 13.77</td>
<td>3.87 4.13</td>
<td>70.3 70.0</td>
</tr>
<tr>
<td>202</td>
<td>1</td>
<td>19.10 18.82</td>
<td>4.86 4.64</td>
<td>74.4 75.4</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>19.42 19.07</td>
<td>4.94 5.50</td>
<td>73.9 71.2</td>
</tr>
<tr>
<td>Difference</td>
<td></td>
<td>0.35 NS²</td>
<td>0.56 NS²</td>
<td>2.7 NS²</td>
</tr>
</tbody>
</table>

¹Nonfat dry weight basis.

²P > .05.
300°F, or between collagen decrease from the raw for the roasts cooked at either oven temperature.

Correlation coefficients for tenderness measures and alkali insoluble collagen content of the cooked tissue and for meat tenderness with percent change of the alkali insoluble collagen content from the raw to the cooked tissue are presented in Table 7. In all cases the values were nonsignificant. Although there was a significant increase in collagen solubility in dilute alkali associated with cooking at either oven temperature (Table 8), no relationship between the increased tenderness of the semimembranosus of top round roasted at 200°F and changes in the collagen solubility characteristics in dilute alkali could be found.

The results are difficult to interpret in view of the contrasting results previously reported concerning the alkali insoluble collagen of beef. Husaini et al. (1950) found a negative correlation between tenderness scores of the cooked tissue and the alkali insoluble protein content of the raw tissue. Correspondingly, Loyd et al. (1959) reported a negative correlation between the alkali insoluble protein content of the raw tissue and tenderness of the cooked tissue. McClain et al. (1965), on the other hand, indicated that the alkali insoluble collagen content of cooked muscles was not related to shear values of the cooked muscles. Similar results were found by Smitherman et al. (1969) in semimembranosus muscle roasted at 200° and at 300°F. Results indicated that the amount of alkali insoluble collagen of the cooked tissue was not related to shear values or tenderness scores of the cooked muscle.
Table 7. Correlation coefficients of alkali insoluble collagen measures and meat tenderness.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>r values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkali insoluble collagen content vs (mg collagen/g cooked tissue&lt;sup&gt;1&lt;/sup&gt;)</td>
<td></td>
</tr>
<tr>
<td>Shear value</td>
<td>0.012 NS&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tenderness score</td>
<td>-0.021 NS&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chew count</td>
<td>0.100 NS&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Percent decrease in alkali insoluble collagen&lt;sup&gt;1&lt;/sup&gt; with heating vs</td>
<td></td>
</tr>
<tr>
<td>Shear value</td>
<td>0.452 NS&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tenderness score</td>
<td>-0.458 NS&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chew count</td>
<td>0.461 NS&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>Nonfat dry weight basis.

<sup>2</sup>P > .05.
Table 8. Student's t-test values for alkali insoluble and Ringer insoluble collagen content between the raw and cooked samples.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>t values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkali insoluble collagen content$^1$ of raw tissue with</td>
<td></td>
</tr>
<tr>
<td>tissue cooked at 200°F</td>
<td>8.085**</td>
</tr>
<tr>
<td>tissue cooked at 300°F</td>
<td>10.925***</td>
</tr>
<tr>
<td>Ringer insoluble collagen content$^1$ of raw tissue with</td>
<td></td>
</tr>
<tr>
<td>tissue cooked at 200°F</td>
<td>0.389 NS$^2$</td>
</tr>
<tr>
<td>tissue cooked at 300°F</td>
<td>1.042 NS$^2$</td>
</tr>
</tbody>
</table>

$^1$Nonfat dry weight basis.

$^2$P > .05.

**P < .01.

***P < .001.
**Ringer Insoluble Collagen**

The Ringer insoluble collagen content on a nonfat dry weight basis of the raw and cooked tissue is presented in Table 9. The roasts cooked at 200°F had significantly less (P < .05) Ringer insoluble collagen than did the roasts cooked at 300°F. This difference can not definitely be attributed to the difference in heat treatments, however, since the Ringer insoluble collagen content of raw samples was significantly less (P < .05) for roasts cooked at 200°F than for those cooked at 300°F.

In contrast to the results obtained with the alkali extraction, no significant difference in the Ringer insoluble collagen content on a nonfat dry weight basis was obtained between the raw and cooked sample for either roasting temperature (Table 9).

Correlation coefficients for tenderness measures with Ringer insoluble collagen content of the cooked tissue, and for tenderness measures with percent change in Ringer solubility of the collagen from the raw to the cooked tissue are presented in Table 10. All correlations were nonsignificant. The data obtained indicated that the increased tenderness of the roasts cooked at 200°F was not related to changes in the collagen solubility in Ringer’s solution.

**Implications**

On the basis of the data obtained, one may conclude that roasting at 200°F did increase the tenderness of semimembranosus muscle but no explanation for the increased tenderness was revealed by studying the
Table 9. Ringer insoluble collagen content of the raw and cooked tissue and percent change in collagen with heating at two oven temperatures.

| Animal number | Pair number | Ringer insoluble collagen |  |  |  |  | Percent change with heating
|               |            | Raw mg collagen/g meat¹ |  |  |  |  | Oven temperature
|               |            | 200°F | 300°F | 200°F | 300°F | 200°F | 300°F |
| 207           | 1           | 29.53 | 30.06 | 27.90 | 31.09 | -5.5  | 3.4   |
| 153           | 2           | 22.06 | 23.01 | 26.19 | 29.35 | 18.7  | 27.5  |
| 153           | 1           | 32.46 | 32.87 | 27.94 | 29.32 | -13.9 | -10.8 |
| 202           | 2           | 19.92 | 20.65 | 24.30 | 24.40 | 22.0  | 18.2  |
| 202           | 1           | 22.96 | 25.29 | 23.92 | 26.30 | 4.2   | 4.0   |
| Mean          |             | 25.39 | 26.38 | 26.05 | 28.09 | 5.1   | 8.4   |
| Difference    |             | 0.99* | 2.04* | 3.3   | NS³   |

¹Nonfat dry weight basis.

²Negative sign indicates decrease.

³p > .05.

*P < .05.
Table 10. Correlation coefficients of Ringer insoluble collagen measures and meat tenderness.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>r values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ringer insoluble collagen content$^1$ vs</td>
<td></td>
</tr>
<tr>
<td>(mg collagen/g cooked tissue)</td>
<td></td>
</tr>
<tr>
<td>Shear value</td>
<td>0.315 NS$^2$</td>
</tr>
<tr>
<td>Tenderness score</td>
<td>-0.296 NS$^2$</td>
</tr>
<tr>
<td>Chew count</td>
<td>0.301 NS$^2$</td>
</tr>
<tr>
<td>Percent change in Ringer insoluble collagen$^1$</td>
<td></td>
</tr>
<tr>
<td>with heating vs</td>
<td></td>
</tr>
<tr>
<td>Shear value</td>
<td>0.555 NS$^2$</td>
</tr>
<tr>
<td>Tenderness score</td>
<td>0.496 NS$^2$</td>
</tr>
<tr>
<td>Chew count</td>
<td>0.404 NS$^2$</td>
</tr>
</tbody>
</table>

$^1$Nonfat dry weight basis.

$^2$P > .05.
relationship of meat tenderness and collagen solubility in dilute alkali or Ringer's solution.

If the increased tenderness of beef obtained with long slow heating is associated with any changes in the collagen molecule, it appears that some measure other than the solubility characteristics will be necessary to reveal the nature of the change. Since heating would not be expected to alter the peptide bonds, some means of measuring changes in the intra- and intermolecular cross-linkages of collagen in muscle tissue might reveal changes that can not be assessed by measuring hydroxyproline.
CHAPTER V

SUMMARY

I. SCOPE OF THE STUDY

Five pairs of top round roasts from three 12 to 16 month old Hereford steers of similar genetic background and feeding regime were used in the study. One roast from each pair was cooked at 200°F and the other at 300°F to an end point of 158°F in the center of the semimembranosus, the test muscle.

The effects of the two heat treatments on cooking time, cooking losses, energy consumption, meat tenderness, and collagen solubility in 0.1 N NaOH and Ringer's solution were studied. Meat tenderness was measured subjectively by sensory scores and chew count by a panel of six judges. The Warner-Bratzler shear provided an objective measure of tenderness. Collagen solubility of the raw and cooked tissues for both oven temperatures was studied by extracting lyophilized samples with dilute alkali and Ringer's solution. The residual amounts remaining after extraction were hydrolyzed, and collagen content measured by analyzing for hydroxyproline. Various collagen measurements were compared with the tenderness measures.

II. PRINCIPAL FINDINGS

Semimembranosus muscles roasted at 200°F had longer cooking times per pound, higher total cooking losses, more energy consumed, and were more tender than pair mates roasted at 300°F. All correlations between
collagen solubility measurements and tenderness measures were nonsignificant.

III. IMPLICATIONS

Roasting at 200°F increased the tenderness of semimembranosus muscle but no explanation for the increased tenderness was revealed by studying the relationship of meat tenderness and collagen solubility in dilute alkali or Ringer's solution. If the increased tenderness of beef obtained with the long slow heating is associated with any changes in the collagen molecule, it appears that some measure other than solubility characteristics will be necessary to reveal the nature of the change.
LIST OF REFERENCES


Cover, S. 1943. Effect of extremely low rates of heat penetration on tendering of beef. Food Res. 8, 388.


Woessner, J. F. 1961. The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. Arch. Biochem. and Biophy. 93, 440.
VITA

The author was born in Ozark, Alabama, on August 11, 1946. From 1952 to 1963 she attended elementary school and high school in Greenville, Alabama. In 1963 her family moved to Pensacola, Florida, where she attended Pensacola High School and was graduated in 1964. The following fall she attended Pensacola Junior College, and in May, 1966, she received her Associate of Science degree in Home Economics. In the fall of 1966 she entered the University of Alabama and was graduated with a Bachelor of Science degree in Home Economics. In the summer of 1968 she accepted a research assistantship at The University of Tennessee and began work toward a Master of Science degree.

She is a member of Phi Upsilon Omicron, Gamma Sigma Epsilon, Phi Theta Kappa, the American Home Economics Association, and the Institute of Food Technologists. She was the recipient of the Borden Award and the American National CowBelles Research Fellowship.

The author is the daughter of Mr. and Mrs. L. C. Whetstone of Pensacola, Florida.