EFFECTS OF FOUR MOIST HEAT TREATMENTS ON COLLAGENOUS CONNECTIVE TISSUE IN BOVINE MUSCLE

by

Julia Leigh Rister

B. S., Texas Tech University, 1975

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Foods and Nutrition

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1976

Approved by:

Dorothy L. Harrison

Major Professor

THIS BOOK
CONTAINS
NUMEROUS PAGES
WITH THE ORIGINAL
PRINTING BEING
SKEWED
DIFFERENTLY FROM
THE TOP OF THE
PAGE TO THE
BOTTOM.

THIS IS AS RECEIVED
FROM THE
CUSTOMER.

TABLE OF CONTENTS

INTRODUCTION

Tenderness plays an important role in consumer acceptance of beef. A number of factors determine beef tenderness with the amount and distribution of connective tissue being one of the factors considered responsible for variation in tenderness. Connective tissue has four major parts: the collagenous, elastic and reticular fibers and the ground substance, with collagen representing the major portion of this moiety (Paul, 1972). Since collagen is a major source of hydroxyproline, specific assays for this amino acid can be used to estimate the quantity of collagenous tissue in bovine muscle (Blackburn, 1968).

Neuman and Logan (1950) developed a method of assay for hydroxyproline that involves its oxidation to pyrrole-2-carboxylic acid, and then formation of a red chromagen on addition of Erlich's reagent (p-dimethylamino benzaldehyde). Numerous modifications of that method have been derived to eliminate variation of results (Wierbicki and Deatherage, 1954; Hutterer and Singer, 1960; Prockop and Udenfriend, 1960; Woessner, 1961; Bergman and Loxley, 1963; Kivirikko et al., 1967; and Blumenkrantz and Asboe-Hansen, 1975). Woessner's (1961) method offers recovery of as little as one part of hydroxyproline in 4000 parts of other amino acids, but Bergman and Loxley's (1963) suggest their method affords greater stability and sensitivity of the chromagen than is obtained with Woessner's method.

Wierbicki and Deatherage (1954) adapted the Neuman and Logan method for measuring the hydroxyproline content of beef muscle. Loyd and Hiner (1959) also modified the Neuman and Logan procedure, and found a negative correlation between collagen content and sensory panel tenderness ratings with r values ranging from -0.695 to -0.903. Parrish et al. (1962) modified the Troll and Cannan (1953) ninhydrin procedure of hydroxyproline determination to study the relation of hydroxyproline content to sensory tenderness of several beef cuts. The coefficient of correlation of the mean hydroxyproline and sensory tenderness values for all cuts examined was -0.843***. Hydroxyproline content was a better measure of the tenderness of less tender steaks than of tender steaks. Paul et al. (1973) studied the extent of solubilization of collagen in semitendinosus (ST) and biceps femoris (BF) muscles when heated to internal temperatures of 58°, 67°,

75° or 82°C. Their data showed a highly significant increase in percentage solubilized collagen with increasing internal temperature. Correlation coefficients for percentage solubilized hydroxyproline vs shear force measurements were 0.09 ns for the ST and -0.45* for the BF. Correlation coefficients for penetrometer readings vs percentage hydroxyproline solubilized were -0.80** for ST and -0.55** for BF. They stated that their results suggest that increasing coagulation of muscle fibers is more important than the breakdown of the collagenous tissue in controlling tenderness changes in the muscles studied. Penfield and Meyer (1975) investigated changes in the connective tissue component of ST cores heated in a water bath at rates comparable to oven roasting of 2 kg top rounds at 93° or 149°C. They observed increased ($P \leq 0.01$) solubilization of hydroxyproline as end point temperature increased. As percentage of hydroxyproline solubilized increased, shear values decreased ($r = 0.704^{**}$). They stated that their results suggest other factors such as endogenous proteolytic enzymes in addition to collagen solubilization are responsible for the increased tenderness of meat cooked by slow heating.

This study investigated (1) effects of four moist heat treatements on collagenous connective tissue in bovine muscle measured by hyroxyproline assay and (2) the relationship of hydroxyproline values to tenderness measurements.

REVIEW OF LITERATURE

Gross structure and composition of muscle tissue

Muscle fiber. Meat is striated, voluntary skeletal muscle. Venable (1963) defined skeletal muscle as a complex package of a great number of variable structures. The contractile cellular unit of skeletal muscle is the long, cylindrical and multinucleated muscle fiber. Diameters of fibers vary within the muscle, between muscles, with age and with degree of activity of the animal (Paul, 1972). The live muscle fiber operates as a physiological unit consisting of many myofibrils, nuclei and inclusions such as mitochondria, glycogen granules and fat droplets embedded in the sarcoplasm (Paul, 1972). Under the light microscope, the muscle fiber demonstrates

both longitudinal striations and cross-striations when fixed properly and stained. Longitudinal striations are formed by parallel arrangement of long thin fibrils; whereas, cross-striations are formed by alternating bands along the length of the myofibril. Bands possessing an affinity for iron hematoxylin stain are called A bands; areas not accepting the stain are called I bands. A narrow area within each I band is stainable and is referred to as the Z ban (Birkner and Auerbach, 1960). The portion of the myofibril bounded by a Z band at either end is considered the basic structural and functional unit, and is designated the sarcomere (Fukuzawa and Briskey, 1970).

Each single muscle fiber is enclosed by extremely thin networks of connective tissue called the endomysium. Muscle fiber bundles of varying size are bounded by larger sheets of connective tissue, the perimysium. The outer layer of connective tissue surrounding the entire muscle is called the epimysium (Cassens, 1971; Meyer, 1968). Connective tissue is distinguished from muscle fiber protein in the chemical laboratory by differences in solubility. Muscle fiber protein is regarded as that portion of total nitrogen-containing material that is soluble in dilute alkali; the insoluble residue is classed as connective tissue (Paul, 1957). The amount of connective tissue proteins in meat is dependent on the anatomical location and physiological function of muscle (Vognarova et al., 1968).

Collagenous connective tissue. Unlike most other tissues, connective tissue has relatively few cells and much intracellular material (Meyer, 1968). The primary components of connective tissue are collagenous, elastic and reticular fibers and the ground substance. Assessment of those tissue components is according to their response to specific treatments such as acid or alkaline solubility, thermal degradation, staining ability and enzymatic substrates (Miller and Kastelic, 1956). The moiety designated as collagen can be separated by autoclaving for conversion of collagen to gelatin. Determination of nitrogen in the resulting hydrolysate constitutes an estimate of collagen content (Hall, 1961; Birkner and Auerbach, 1960). X-ray diffraction methods and electron microscopy for collagen preparations have been used to collect data on the shape and organization of the collagen molecule (Meyer, 1968). Beef collagen has an unusually high percentage (64%) of amino acid residues that have nonpolar side chains

with glycine being especially high (19.9%). The arrangement of types of amino acid residues is believed to be a section of amino acids with nonpolar side chains followed by those possessing polar side chains capable of hydrogen bonding and salt links. Collagens are unusual in their possession of fair amounts (from 5 to 13%) of hydroxyproline (Meyer, 1968).

Elastic connective tissue. Elastin fibers are characterized by their chemical inertness or insolubility; the final residue after exhaustive extraction with dilute acids or alkali is defined as elastin (Hall, 1961). Unlike collagen, it does not undergo hydrolysis during cooking (Meyer, 1968). Elastin fibers branch readily and are extremely elastic. These fibers are abundant in tendons and ligaments, which often are trimmed away before meat is cooked (Meyer, 1968), but are scarce in muscle, with some exceptions such as in bovine semitendinosus (Cassens, 1971). Some study of elastin fiber structure has been done according to the methods applied to collagen. Their structure appears similar to that of linear proteins, but of a different type than that of the collagens. Beef elastin contains 14% more nonpolar amino acids than beef collagen; however, it is similar to collagen in its small content of histidine, cystine, tyrosine, and tryptophan (Meyer, 1968). Collagen appears to contain 50 to 100 times as much hydroxyproline as elastin (Paul, 1972).

Reticular connective tissue. Reticulin, the protein in reticular fibers, closely resembles collagen. Reticular fibers are branched highly and are recognized chiefly by their ability to combine with certain histological stains, notably the silver stains (Hall, 1961). At points where connective tissue is adjacent to other tissues, reticular fibers are present. For example, reticulin is found between the endomysial collagenous fibers and each muscle fiber membrane or sarcolemma (Birkner and Auerbach, 1960).

Ground substance. The ground substance is a homogeneous background material of mucopolysaccharides and mucopolysaccharide-protein complexes in differing degrees of polymerization (Miller and Kastelic, 1956).

Adipose tissue. Some researchers consider adipose tissue a specialized type of connective tissue containing large deposits of fat. Adipose tissue is composed of lipid, water, salts and other compounds commonly present

in cells in small amounts. In addition to deposition in adipose tissue, fat cells also may be scattered through, or in groups, in loose connective tissue (Meyer, 1968).

Effects of collagenous and elastic tissues on tenderness

Correlations for collagenous tissue and tenderness. The amount and distribution of connective tissue is considered a primary factor determining degree of tenderness of a given piece of meat. Attempts to relate quantitative measures of this component of muscle tissue to mechanical and sensory evaluations of tenderness have yielded variable results. A significant negative correlation (-0.87) between connective tissue and tenderness scores of cooked beef was reported by Husaini et al. (1950). Loyd and Hiner (1959) found a negative correlation between collagen and sensory panel ratings with r values ranging from -0.695 to -0.903. Adams et al. (1960) indicated a significant negative association between collagen content and meat tenderness. Parrish et al. (1962) reported a highly significant negative correlation (-0.843) between mean hydroxyproline values and sensory tenderness values. A highly significant, but only moderate correlation (-0.54**) between collagen in raw meat and panel scores for tenderness of connective tissue was reported by Kim et al. (1967). Cross et al. (1973) found that percentage soluble collagen was related significantly to sensory panel ratings for amount of connective tissue.

McClain et al. (1965) found that the amount of collagen in raw or cooked beef muscle was not related to shear values of the cooked meat. Bayne et al. (1971) reported that the alkali insoluble collagen content of cooked meat was not related to shear values, but it was related to tenderness scores ($r = 0.657^*$) and chew count ($r = 0.643^*$). Paul et al. (1973) found correlation coefficients for the percentage solubilized hydroxyproline and shear force measurements of 0.09 ns in the ST and -0.45^* in the BF.

Age related changes in collagen. The toughness of meat from older animals often is attributed to increasing amounts of connective tissue (Paul, 1972); however, more collagen occurs in lean tissue of the young animal than in that of older age groups (Goll et al., 1963; Vognarova

et al., 1968). After the animal is mature there is little quantitative change in percentage of connective tissue (Paul, 1972). Goll et al. (1964) reported that maturation of collagen was accompanied by formation of strong cross linkages within the tropocollagen molecule. They suggested that the number and strength of the cross-links in collagen may play an important role in meat tenderness. Conceivably, the formation of cross linkages could alter the amount of collagen converted to gelatin during cooking, which ultimately would affect meat tenderness (Goll et al., 1964). Verzar (1964) reviewed the release of hydroxyproline during thermal contraction. He concluded that the quantity of hydroxyproline released declined with age. Verzar called the fraction that is solubilized on heating labile collagen. He suggested that the decrease of labile collagen with age can be explained on the basis of increased cross-linking of collagen with maturation. Vognarova et al. (1968) found that less collagen is solubilized during cooking meat from old than from young animals. Herring et al. (1967) reported that decreased yields of labile collagen were correlated with increased toughness in cooked meat from animals of widely different age range. Field et al. (1970a) found that heat labile collagen from both intramuscular and epimysial tissue was higher for tender than for tough muscles. Results suggest that tenderness increases with amount of heat labile collagen in the muscle.

Correlations for elastic tissue and tenderness. Elastin fibers are relatively inert and may be affected slightly by heat, but not enough to influence the tenderness of the tissue (Paul, 1972). Cross et al. (1973) observed that elastin concentration was not related consistently to tenderness variation of bovine muscle.

Other factors affecting muscle tenderness. Many interrelated factors contribute to the tenderness or toughness of meat. Those factors include breeding, feeding, age, post-mortem changes, presence of collagenous and elastic fibers, size of fibers, method of cooking and probably many others (Hiner, 1955; Paul, 1972). Two structural components, muscle fibers and connective tissue, are involved in meat tenderness (Paul, 1957, Ritchey et al., 1963). Varied results for the relationship between connective tissue and tenderness may be attributed to differences in cooking methods, age, muscles used, end point temperatures studied, method of measuring connective

tissue and the fact that meat is not a simple one component system (Cover et al., 1962; Harrison, 1975).

Heat induced changes in connective tissue

Marked changes in meat during heating may be attributed to denaturation and coagulation of proteins, loss of water holding capacity, melting of fat, alterations in pH and chemical changes in heat labile compounds. Type and extent of changes induced by heating vary with meat composition, duration of heating and internal temperature reached (Paul, 1972).

Solubilization of collagenous tissue. In studies on changes in connective tissue, alkali insoluble collagen decreased during heating (Winegarden et al., 1952; Griswold, 1955; Irwin and Cover, 1959; Cover et al 1962a; Ritchey and Cover, 1962; Ritchey et al. 1963; Yang and Couvillion, 1964; McClain et al., 1965; Bayne et al., 1971; Cross et al., 1973; Paul et al., 1973; Penfield and Meyer, 1975). In studies on longissimus dorsi (LD) and biceps femoris (BF) muscles the collagen content decreased with increasing degrees of doneness (Cover et al., 1962a; Ritchey and Cover, 1962; Ritchey et al., 1963). Collagen content was greater in BF than in LD in raw steaks and in steaks cooked to final internal temperatures of 61° or 80°C (Cover et al., 1962a; Ritchey et al., 1963), but there was little difference between the two muscles at an internal temperature of 100°C (Cover et al., 1962a). Rates of conversion of collagen to gelatin were similar in the two muscles (Ritchey et al., 1963).

A highly significant increase in percentage solubilized collagen with increasing internal temperature in both ST and BF muscles was reported by Paul et al. (1973). Penfield and Meyer (1975) evaluated changes in beef collagen in ST heated at two rates. They reported that with slow heating the higher the internal temperature, the greater the solubilization of hydroxyproline containing materials. However, Bayne et al. (1971) using semimembranosus (SM) muscle found that the amount of hydrolyzed collagen did not change with different heating rates.

An increase in collagen content of bavine LD and Sm muscles with cooking was reported by Skelton et al. (1963). Harrison et al. (1953) obtained higher collagen nitrogen values for cooked than for raw samples

from rib roasts, loin steaks and round steaks.

Shrink temperature of collagenous tissue. Collagen is denatured at its shrink temperature, Ts. Lawrie (1968) gave the Ts of collagen in intact muscle as 60°C. Field et al. (1970b) used differential thermal analysis to study Ts of collagen prepared from intra- and inter-muscular connective tissue from LD and BF muscles of mature cows. Collagen from the LD melted at a lower ($P \leq 0.05$) temperature than that from BF. Peak melting point of intramuscular collagen was 65.9°C for LD and 66.3°C for BF. Differences in peak melting points between epimysial and intramuscular collagen were significant ($P \leq 0.01$). Data on thermal characteristics of intramuscular collagen should not be interpreted to apply to epimysial collagen. The Ts of collagen occurs over a narrow temperature range regardless of age or anatomical location (Field et al., 1970b). In conversion of collagen to gelatin temperatures higher than the Ts or treatment with denaturing chemicals, is required to rupture the cross-links that hold the collagen chains together (Paul, 1972).

Machlik and Draudt (1963) studied the effect of heating time and temperature on the shear value of beef SM. They noted little change in shear value up to 50°C, a marked decrease between 50° and 60°C, an increase in shear value from 60° to 70°C and some decrease at about 75°C. They attributed the initial decrease in shear value to collagen shrinkage, the increase in shear value between 60° and 70°C to hardening of the contractile fibers and the subsequent decrease in shear value at 75°C to collagen transformation to gelatin.

Microscopic changes of collagenous tissue. Changes in collagenous tissue induced by heat may be followed histologically. As collagen is heated, the color taken up from the histological stains is altered (Paul, 1972). Skelton et al. (1963) observed a heat induced change in the organization of collagen from the fibrous to the granular stage. Reid and Harrison (1971) did histological studies on raw and cooked SM muscle, and observed a greater quantity of total connective tissue in cooked than in raw muscle samples.

Changes in elastic tissue. Lawrie (1968) found elastin to be quite resistant to heat change in the temperatures normally used for cooking meat, but Winegarden et al. (1952) suggested that elastin softens similarly to

collagen, however not to the same extent.

Effect of rate of Heat penetration on tenderness

Softening of connective tissue. The conductivity of heat through meat is influenced by several factors including the cooking medium and temperature; the size and shape of the sample; the amount of lean, fat, connective tissue and bone in the sample; the characteristics of the meat surface and the changes induced in the meat by heat such as protein denaturation, loss of water and melting of fat (Paul, 1972). For several years investigators have reported that tenderness may be improved by long-time, low-temperature cooking (Cover, 1943; Bramblett et al., 1959; Hood, 1960; Laakkonen et al., 1970a; Bayne et al., 1971; Penfield and Meyer, 1975). The toughening effect on muscle fibers and the softening effect on connective tissue during cooking are dependent on both time and temperature with the time factor being more important for collagen softening and the temperature factor more important for muscle fiber toughening (Weir, 1960).

Cover (1943) studied the effects of rate of heat penetration on tenderness of beef roasts. Paired standing rib and arm bone chuck were cooked well-done, and bottom round roasts were cooked rare or well-done at oven temperatures of 80° or 125°C. She found that well-done chuck and round roasts were more tender when roasted at 80°C than at 125°C. When the rate of heat penetration was slow enough so that at least 30 hours were required for the roasts to lose their pink color, they were always tender. Improved tenderness was related to the slow release of the water of hydration allowing for the effective conversion of collagen to gelatin.

Bramblett et al. (1959) cooked beef rounds wrapped in aluminum foil at 63°C for 30 hours and at 68°C for 18 hours. Meat cooked at 63°C was more tender ($P \leq 0.01$) than that cooked at 68°C. A highly significant negative correlation (-0.73**) was found between shear values and length of time the meat was held at an internal temperature of 57° to 60°C. The increased tenderness was attributed to softening of collagenous tissue and retarded toughening of the myofibrillar proteins occuring at that temperature interval.

Hood (1960) cooked beef shoulder roasts in open pans and in aluminum

foil at an oven temperature of 149°C to an end point temperature of 77°C. Roasts wrapped in foil were less tender than those cooked in open pans as a result of a more rapid rate of heat penetration in foil wrapped roasts.

Bayne et al. (1971) cooked roasts from Sm muscle at 93° or 149°C to an end point of 70°C. Roasts cooked at 93°C were significantly more tender than those cooked at 149°C as indicated by shear value and sensory panel evaluation.

Shaffer et al. (1973) cooked top round roasts from the frozen state by dry or moist heat at 177° or 205°C to internal temperatures of 60°, 70° or 80°C. The internal temperature of roasts cooked by moist heat increased more rapidly than the internal temperature of roasts cooked by dry heat. Tenderness was not affected significantly by oven temperature (Shaffer et al., 1973).

A study comparing microwave energy with conventional energy showed more solubilization of collagen in meat cooked in the microwave oven (McCrae and Paul, 1974). They used steaks from ST muscles heated by four methods: microwave, oven broiling, braising and roasting, All samples were heated to an internal temperature of 70°C except the braised steaks, which were heated to 98°C and held at that temperature for 30 minutes. Rate of heat penetration for the different heating methods in order of decrease in rate was: microwave heating, oven broiling, braising and roasting. Higher values for hydroxyproline solubilized using the microwave heating indicates that the microwave energy influences the collagen solubilization in a different manner than does conventional energy. There were no significant differences among different heating rates produced by the three conventional heating methods. They suggested that the way in which energy is supplied alters the solubilization of collagen more than the rate of heat penetration (McCrae and Paul, 1974).

Collagenase activity. Laakkonen et al. (1970a) devised a model system to study the relationships between the tenderness of slowly cooked meat and its water-holding capacity, pH and the amount of water-soluble components. Muscle sample analyses was done at 1-hour intervals between the 3rd and 10th hour of heating. Between the 4th and 6th hour when the meat was in the 50° to 60°C temperature interval the major decrease in shear value was observed. At internal temperatures below 60°C the collagenase enzyme in

the meat was still active but above 70°C the enzyme was inactivated (Laakkonen, 1970b). They suggested that the reduced rate of heating at low oven temperatures may promote an environment conducive to collagenase activity thereby promoting tenderization.

Penfield and Meyer (1975) used ST cores heated to 40°, 50°, 60° or 70°C in a water bath at rates comparable to oven roasting of 2 kg top rounds at 93° and 149°C. Changes in tenderness were measured by the Warner-Bratzler shear. Cores heated at the more rapid rate of heat penetration were less tender ($P \leq 0.05$) than those heated at the slow rate. The greatest decrease in shear values occurred between 50° and 60°C. An increase in enzymatic activity also was apparent at that temperature interval suggesting that differences in tenderness attributable to heating rate may result in part from differing degrees of collagenase activity (Penfield and Meyer, 1975).

Effect of cooking method on tenderness

Cooking methods have been classified as dry or moist heat cookery depending on the atmosphere surrounding the meat. Some examples of moist heat cookery include wrapping in aluminum foil, cooking in oven film bags, braising, steaming, pressure cooking and cooking in a slow cooker. Methods of dry heat cookery include broiling, roasting and deep fat frying. Steam is a more efficient conductor of heat than dry air; therefore, meat cooked by moist heat methods cooks at a faster rate. Liquid fat, a dry heat method, conducts heat better than dry air. The microwave oven provides a dry atmosphere in which energy is supplied to the meat as high-frequency electromagnetic waves. Microwave cookery is the fastest method of meat cookery (Paul, 1972).

Customarily dry heat methods have been recommended for "tender" cuts containing relatively small amounts of connective tissue and moist heat methods for "less tender" cuts that contain a greater proportion of connective tissue (Paul, 1963).

Changes in connective tissue. The moisture provided by moist heat cookery has been considered necessary for softening of collagenous connective tissue. Cover (1941) questioned the necessity of added moisture since meat itself contains about 70° water. She cooked top and bottom

round roasts to an internal temperature of 80°C in water at 90°C and in an oven at 90°C and found that roasts cooked by the dry heat method were more tender than those cooked by moist heat. Roasts cooked by dry heat required 26 hours; whereas, those cooked in moist heat required 2 hours. The slower rate of heat penetration allowed for effective tendering of the cuts containing much connective tissue (Cover, 1941).

Irwin and Cover (1959) broiled steaks from BF and LD muscles to an internal temperature of 61°C. The loss of about 25% of the collagen nitrogen during broiling indicates that some collagen is solubilized using dry heat. Moisture in the muscle probably was sufficient to provide an atmosphere conducive to collagen hydrolysis.

Hood (1960) cooked roasts from U. S. Good or U. S. Standard triceps brachii muscles (a muscle rated as "medium tender") by dry or moist heat at an oven temperature of 300°F to an end point of 170°F. Roasts cooked by dry heat were more tender than those cooked by moist heat as evaluated by tenderness scores and shear values.

Shaffer et al. (1973) in an experiment described earlier in this paper noted that top round roasts cooked by dry heat were scored more tender ($P \leq 0.05$) than those cooked by moist heat.

Shock et al. (1970) cooked pieces of SM muscle by two dry heat treatments (oven-roasting, deep-fat frying) and two moist heat treatments (oven braising, pressure braising) to an internal temperature of 70°C. Shear values and tenderness scores were not affected significantly by treatment.

Ferger et al. (1972) reported no differences in tenderness attributable to heat treatment for rolled leg of lamb roasts and beef rib roasts cooked from the frozen state by dry and moist heat. Both cuts studied by Ferger et al. (1972) are considered "tender."

McCrae and Paul (1974) in an experiment previously described, found no significant difference in shear force measurements among the various heat treatments.

Cover and Smith (1956) cooked loin or bottom round steaks well-done by broiling or braising. Steaks from the BF muscle were more tender braised than broiled; whereas, steaks from LD showed no significant difference in tenderness when cooked by the two methods.

Tenderness seems to vary with the muscle being studied and the rate at which energy is supplied to the muscle. Cover et al. (1957) noted that LD becomes dryer and harder with extended cooking; whereas, BF becomes increasingly tender when cooked extended periods of time.

Changes in muscle fiber. Cooking causes a decrease in muscle fiber diameter (Satorius and Child, 1938); the contractile fibers shrink in length and width and the sarcomeres become shorter (Paul, 1972). Hostetler and Landmann (1968) studied muscle fiber fragments heated on the stage of a microscope from room temperature to 80°C. Decrease in width of the muscle fiber was most rapid between 45° and 62°C. Shrinkage in length occurred to the greatest extent between 55° and 65°C, but continued slowly as the temperature increased to 80°C. The shrinkage of the fiber proteins caused by denaturation and coagulation results in hardening of the muscle (Cover, 1962b).

MATERIALS AND METHODS

Four beef top rounds (approximately 9 kg) were obtained from a local wholesale meat company. The external fat covering was removed, the semimembranosus (SM) and adductor (AD) muscles were squared off, and divided into eight steaks (Fig. 1). Individual steaks were wrapped in aluminum foil (guage 0.0015), frozen in an upright household freezer at an average temperature of -26.5°C ± 1.5°C (-16°F ± 2°F) and stored in the same freezer until used (2 to 6 weeks).

Treatment combinations were:

Treatment	Oven temperature, °C	Doneness (Internal temperature, °C)
1	94	70, Medium-done
2	94	80, Well-done
3	149	70, Medium-done
4	149	80, Well-done

The experimental design for cooking was a randomized complete block with eight replications. One block (replication) consisted of four oven temperature-end point temperature combinations (Table 1). Steaks were assigned to replications by position within the top round (Fig. 2). The four inside steaks (B,F,C,G) weighed 270-403 g and comprised the odd replications. The four

Fig. 1-Photograph of trimmed top round cut into steaks.

THIS BOOK CONTAINS SEVERAL DOCUMENTS THAT ARE OF POOR QUALITY DUE TO BEING A PHOTOCOPY OF A PHOTO.

THIS IS AS RECEIVED FROM CUSTOMER.

THIS BOOK CONTAINS NUMEROUS PICTURES THAT ARE ATTACHED TO DOCUMENTS CROOKED.

THIS IS AS RECEIVED FROM CUSTOMER.

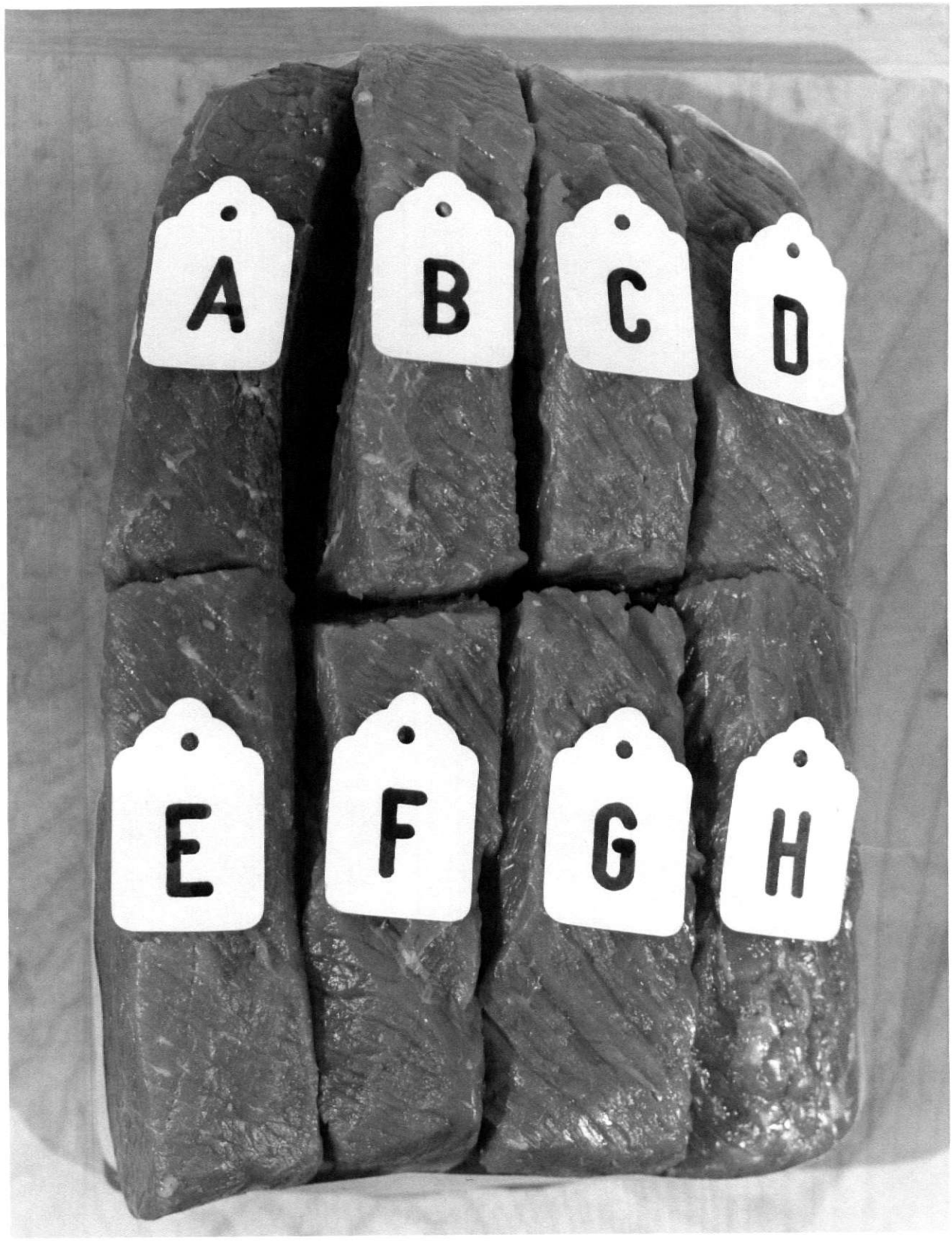

A
B
C
D
E
F
G
H

Table 1-Experimental design for cooking and position of raw sample

Cooking period	Replication	Round	Steak position	Treatment[a]
1	1	1	B	III
			F	II
			C	I
			G	IV
			I	V
2	2	1	A	IV
			E	III
			D	I
			H	II
			J	V
3	3	2	B	I
			F	IV
			C	III
			G	II
			I	V
4	4	2	A	IV
			E	III
			D	II
			H	I
			J	V
5	5	3	B	IV
			F	II
			C	I
			G	III
			J	V
6	6	3	A	IV
			E	I
			D	II
			H	III
			I	V
7	7	4	B	II
			F	I
			C	IV
			G	III
			I	V

Table 1-(concluded)

Cooking period	Replication	Round	Steak position	Treatment[a]
8	8	4	A	IV
			E	I
			D	II
			H	III
			J	V

[a]Treatments randomly assigned

I. 94°C oven temperature, 70°C end point temperature
II. 94°C oven temperature, 80°C end point temperature
III. 149°C oven temperature, 70°C end point temperature
IV. 149°C oven temperature, 80°C end point temperature
V. Raw

outside steaks (A,D,E,H) weighed 206-365 g and comprised the even replications. Selected measurements were made on raw samples. Strips I and J (1.3-cm thick, midway between the proximal and distal ends and across the top round from anterior to posterior) were used for raw sample analysis and were assigned randomly to the two blocks from each round (Table 1, Fig. 2). There were eight evaluation periods with four steaks cooked at each period (Table 1).

Before each cooking period four steaks were defrosted 3 hr at room temperature (approximately 30°C, 78°F) and 20 hr in a refrigerator (approximately 4°C, 40°F). Each steak was placed in an oven film bag (Reynold's Brown-In-Bag - nylon 66 with a heat stabilizer) and closed with a twister tie. Six slits (approximately 5.8-cm long) were made in each bag to allow steam to escape and prevent the bag from breaking. A thermometer (-20°C to 105°C) with a small bulb was inserted through the oven film bag into the center of each steak. The entire system was placed on a low rack in a shallow roasting pan, and cooked in a rotary hearth oven at 94°C (200°F) or at 149°C (300°F) to an internal temperature of 70°C (158°F) or 80°C (176°F) (Fig. 3). Cooked steaks were sampled according to Figure 4.

Fig. 2-Sampling plan for beef top round (SM and AD muscles). Steaks A - H were used for cooked sample analysis. Strips I and J were used for raw sample analysis.

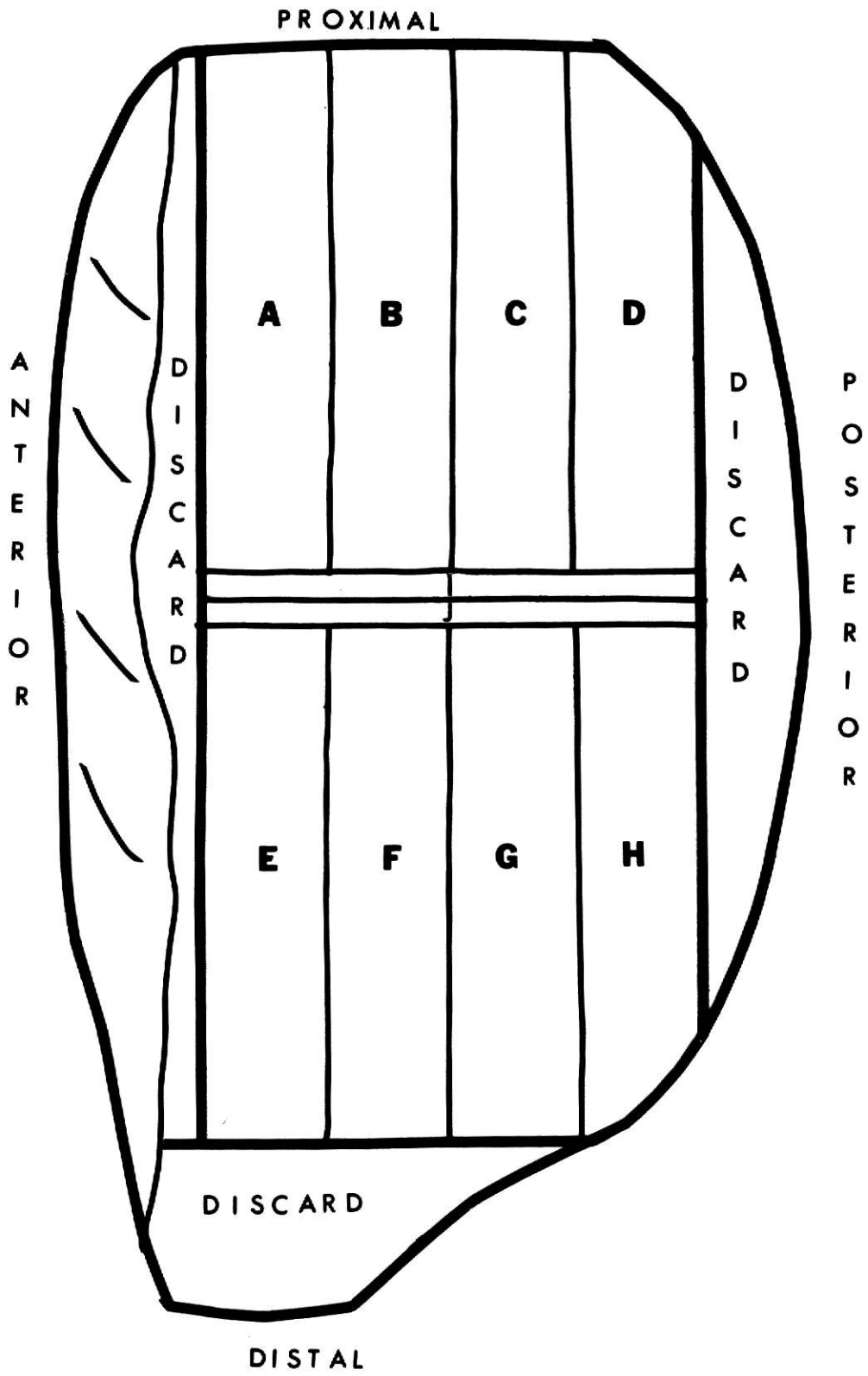

PROXIMAL
ANTERIOR
DISCARD
A
B
C
D
DISCARD
POSTERIOR
E
F
G
H
DISCARD
DISTAL

Fig. 3-Steak in oven film bag prepared for cooking.

Fig. 4-Sampling plan for cooked steaks.

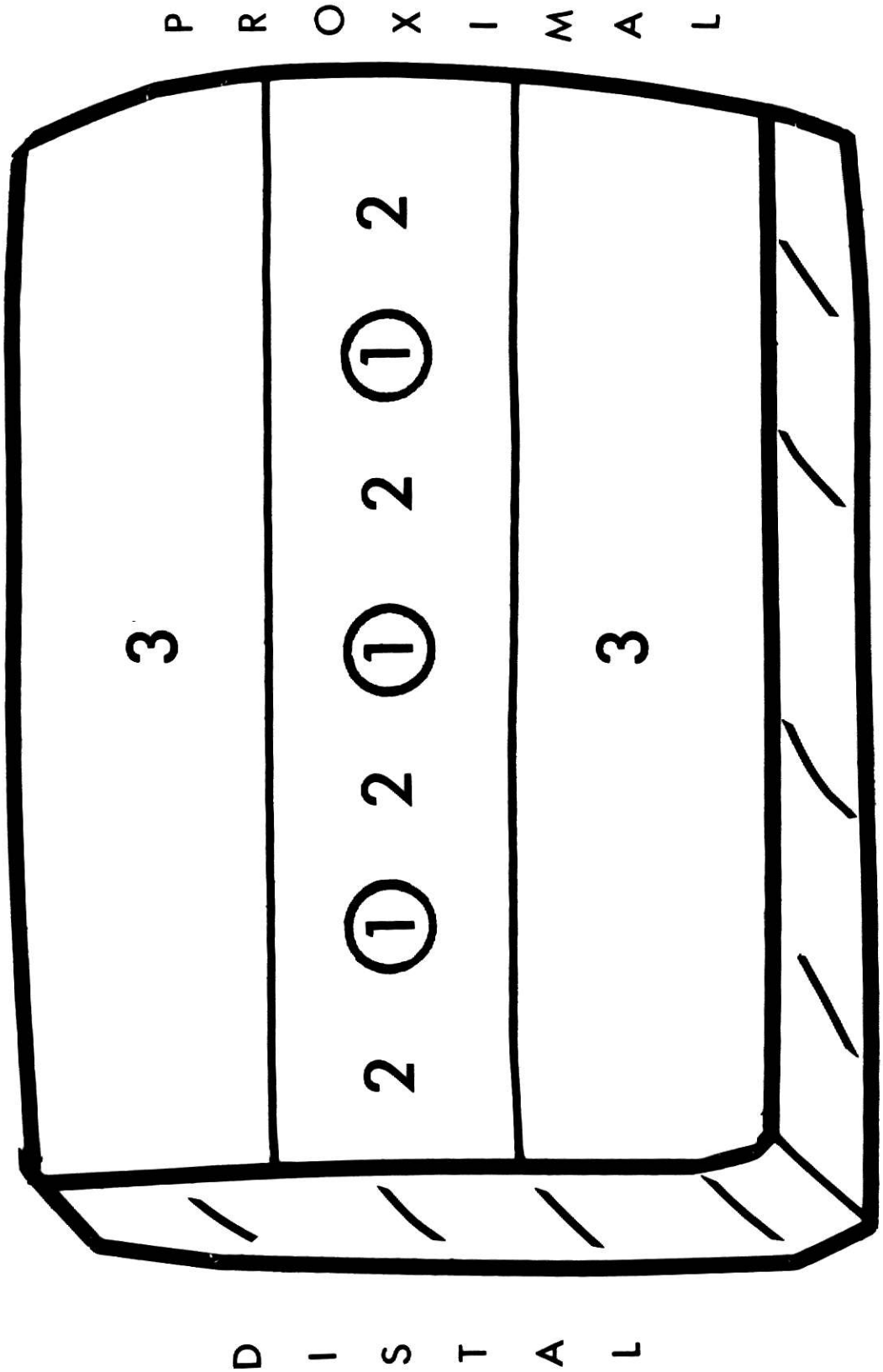
1--Shear cores

2--Hydroxyproline, total moisture and ether extract measurement

3--Sensory evaluation samples

THIS BOOK CONTAINS NUMEROUS PAGES WITH DIAGRAMS THAT ARE CROOKED COMPARED TO THE REST OF THE INFORMATION ON THE PAGE.

THIS IS AS RECEIVED FROM CUSTOMER.


PROXIMAL
3
2
1
2
1
2
1
2
1
2
3
DISTAL

Rate of heat penetration, cooking time and losses

The rate at which heat penetrated the muscle was measured by recording the time required to raise the internal temperature from the initial temperature to 5°C and every 5°C rise thereafter to the end point temperature. Total cooking time, in minutes, was recorded. Percentages of total and drip cooking losses, based on weight of the thawed raw steak were calculated. Drippings were collected in 100-ml graduated cylinders and allowed to stand 45 min before reading the volume.

Hydroxyproline determination

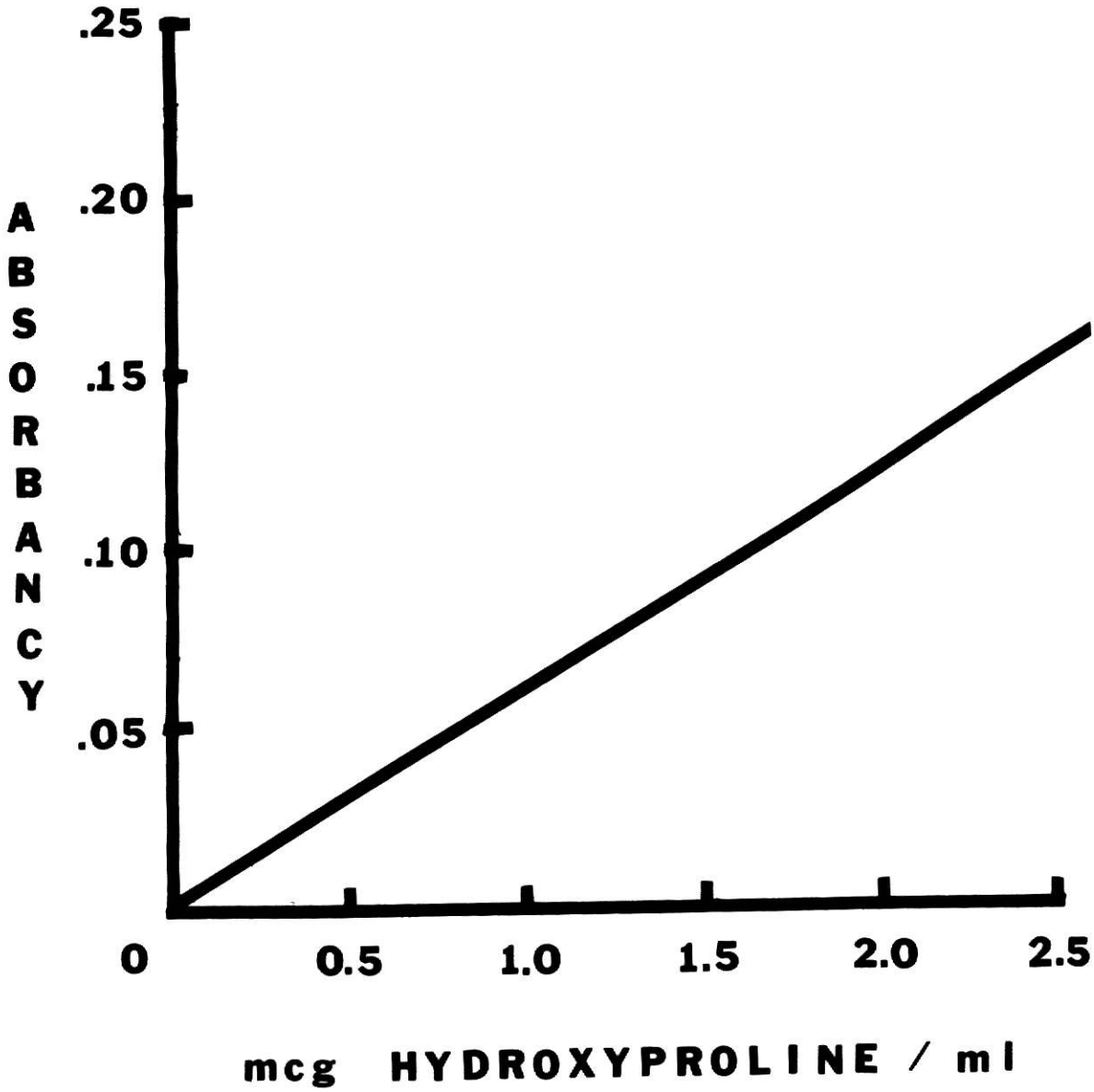
Duplicate analyses of hydroxyproline content were made for both raw and cooked samples. Raw sample preparation was done according to the method outlined by Woessner (1961) with time and temperature modifications suggested by Stegemann and Stalder (1967). Cooked samples were prepared according to the method outlined by Penfield and Meyer (1975). Hydroxyproline assay was done according to the procedure outline by Bergman and Loxley (1963). Details of those methods are given in the Appendix p. 54 to 57. A series of standards was prepared containing 0 to 2.5 mcg L-hydroxyproline to establish a standard curve (Fig. 5). Hydroxyproline values for samples analyzed were determined directly from the standard curve by plotting absorbancy and reading mcg hydroxyproline/ml.

Sensory evaluation

Tenderness, softness and mealiness of 1.3-cm cubes of muscle were evaluated by an eight member laboratory panel using a 7- to 1- point intensity scale (Form I, Appendix, p. 58). Instructions for evaluation (Form II, Appendix, p. 59) were given to panel members during preliminary work. Each panel member standardized his tenderness scores by counting the number of chews necessary to masticate a cube of meat completely. Cubes were presented to panel members in the top of half-pint double boilers set over hot (approximately 51°C, 114°F) water and the entire system placed on an electric hot tray at low heat (71°C $\pm$ 6°C, 160°F $\pm$ 10°F). All sensory evaluation took place within 30 minutes after preparation of the samples.

Fig. 5-Standard curve prepared from averages of a series of standards containing 0 - 2.5 mcg L-hydroxyproline. Absorbance at wavelength 558 was plotted vs. amount of hydroxyproline.

STANDARD CURVE



Shear values

Tenderness was measured by shearing 1.3-cm cores from cooked muscle on a Warner-Bratzler shearing apparatus with a 25-lb dynamometer. Cores were taken from the proximal, center, and distal position in each steak (Fig. 4). Triplicate measurements were made on each core and the over-all shear value was the average of the three shear cores.

Total moisture and ether extract analysis

Percentages of total moisture and ether extract of both raw and cooked samples were measured in the analytical laboratory of the Department of Animal Science and Industry using a modified AOAC method (AOAC, 1976). One inch sterile cotton squares and Soxhlet paper extraction thimbles were defatted by an ether wash, dried well, and weighed. Meat (0.5 g) was smeared on cotton squares, rolled and placed in an extraction thimble. The meat was dried for 16 hours at 95°C, weighed when cool, and then total moisture was calculated.

The thimbles were placed in a Goldfisch Extraction Apparatus and meat samples were extracted overnight. Tared beakers also were placed in the apparatus. The ether was recovered leaving fat in the flask. The beakers were put in a vacuum oven at 95°C for 1 hour. After cooling they were weighed and the percentage ether extract was calculated.

pH

Slurries of both raw and cooked meat were prepared by blending 10 g ground muscle with 100 ml of distilled, deionized water for 2 min in a Waring blendor at high speed. The Slurry was placed in a beaker and the temperature brought to 25°C (77°F). After stirring 30 sec with a magnetic stirrer, the pH was determined on a Beckman expanded scale pH meter. The beaker was rotated 180° and stirred for another 15 sec, after which a second pH reading was made. The pH meter was standardized at 25°C against a buffer of pH 6.86.

Analysis of data

Data for each measurement used to evaluate the cooked steaks were analyzed by analysis of variance:

Source of variation	D/F
Replication	7
Treatment combinations	
Oven temperature (a) (94°C vs 149°C)	1
End point temperature (b) (70°C vs 80°C)	1
Interaction (a x b)	1
Error	21
Total	31

Correlation coefficients were calculated within each treatment combination for selected paired variates.

Data for three measurements (pH, total moisture, ether extract) used to measure characteristics of both raw and cooked meat were analyzed by analysis of variance:

Source of variation	D/F
Replication	7
Raw vs cooked	1
Treatment combinations	1
Oven temperature (a) (94°C vs 149°C)	1
End point temperature (b) (70°C vs 80°C)	1
Interaction (a x b)	1
Error	28
Total	39

RESULTS AND DISCUSSION

Initial weight of steaks, rate of heat penetration

Analysis of variance indicated no significant difference in weights of steaks assigned to the two oven temperatures (Table 2) or to the end point temperatures (Table 3). However, significant ($P \leq 0.01$) differences in weight of steaks attributable to replication (position of steak in the top round, Fig. 1) did occur (Table 8, Appendix, p.60). Variation of steak weights among replications was expected, because weights of top round varied from 7.9 to 9.2 kg, and depth of steaks varied according to position in the top round. Generally, steaks from the even replications weighed less than those from the odd replications.

Data for the rate heat penetrated the muscle are presented in Table 4 and Fig. 6. The time required to raise the internal temperature of the steaks from the initial temperature to 5°C was not significantly different between oven temperatures of 94° or 149°C. From 5°C to an end point temperature of 70° or 80°C, at each 5°C interval, the rate at which heat penetrated the steaks differed ($P \leq 0.001$) between the two oven temperatures. The internal temperature of the steaks cooked at 149°C increased more rapidly than the internal temperature of those cooked at 94°C.

Heat penetrated steaks cooked at 149°C at a fairly constant rate, but rate of increase slowed down slightly for steaks cooked 94°C after the internal temperature reached 50°C. Vollmar et al. (1976) cooked top round roasts in an oven film bag (94°C) or in a slow cooker (85°C) and found that the penetration rate remained fairly constant for both moist heat cooking methods throughout the cooking period.

Oven temperature

Data for effects of oven temperature are presented in Table 2. Total cooking time ($P \leq 0.001$), drip cooking losses ($P \leq 0.001$) and softness score ($P \leq 0.05$) were greater for steaks cooked at 94°C than at 149°C. Drip losses at oven temperature 94° and 149°C can be observed in Figures 7 and 8. In general, the amount of coagulum formed was greater for steaks cooked at 94°C than at 149°C.

Table 2-Means and F-values for selected measurements by oven temperature[a]

Measurement	Oven temp., °C 94°	149°	Difference	F-value
Initial weight, g	316.6	314.8	1.8	0.02
Initial temp., °C	2.4	0.3	2.1	11.07**
Total cooking time, min	91.0	41.3	49.7	374.44***
Cooking losses, %				
Total	32.1	32.0	0.1	0.02
Drip	30.7	26.0	4.7	18.47**
Total moisture, %	61.0	60.9	0.1	0.02
Ether extract, %	5.3	5.4	0.1	0.08
pH	5.3	5.2	0.1	0.12
Shear value, kg/1.3-cm core	2.9	3.2	0.3	2.27
Hydroxyproline solubilized, %	1.6	1.6	0.0	0.11
Sensory scores[b]				
Tenderness	4.9	4.7	0.2	1.55
Softness	4.6	4.0	0.6	6.03*
Mealiness	4.6	4.3	0.3	2.50

[a]Includes data for both end point temperatures

[b]7-(extremely tender, extremely soft, extremely mealy);
1-(extremely tough, extremely hard, extremely chewy)

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$

Table 3-Means and F-values for selected measurements by end point temperature[a]

Measurement	End point temp., °C		Difference	F-value
	70°	80°		
Initial weight, g	312.8	318.6	5.8	0.19
Initial temp., °C	1.8	0.9	0.9	1.70
Total cooking time,	58.5	73.8	15.3	35.18***
Cooking losses, %				
Total	29.5	34.6	5.1	24.00***
Drip	26.8	30.0	0.2	8.68**
Total moisture, %	62.4	59.5	2.9	21.01***
Ether extract, %	4.9	5.8	0.9	3.29
pH	5.3	5.2	0.1	2.06
Shear value kg/1.3-cm core	3.1	3.0	0.1	0.18
Hydroxyproline solubilized, %	1.5	1.8	0.3	2.54
Sensory scores[b]				
Tenderness	5.0	4.6	0.4	9.45**
Softness	4.7	4.0	0.7	8.29**
Mealiness	4.3	4.6	0.3	2.11

[a]Includes data for both oven temperatures

[b]7-(extremely tender, extremely soft, extremely mealy);
1-(extremely tough, extremely hard, extremely chewy)

**P ≤ 0.01; ***P ≤ 0.001

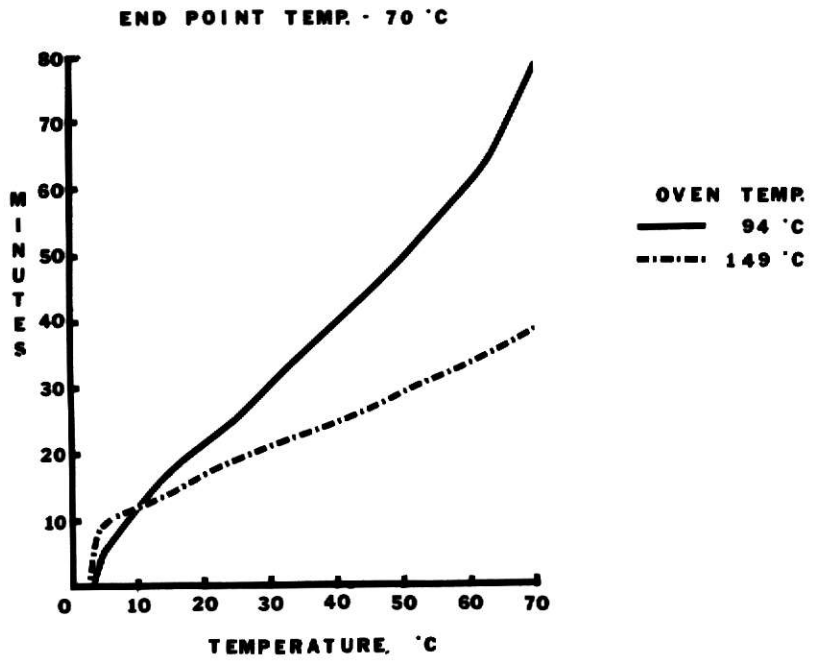
Table 4 -Means and F-values for rate of heat penetration in minutes to reach 5°C and to increase every 5°C to 80°C[a]

Temperature interval, °C	Oven temperature, °C		F-value
	94°	149°	
Initial temp., to 5°C	7.1	8.6	0.92
5° to 10°C	6.1	3.0	36.56***
10° to 15°C	4.8	2.3	72.79***
15° to 20°C	4.5	2.1	95.96***
20° to 25°C	3.9	2.1	81.46***
25° to 30°C	4.2	2.1	56.47***
30° to 35°C	4.3	2.1	68.96***
35° to 40°C	4.6	2.1	120.00***
40° to 45°C	4.8	2.1	205.44***
45° to 50°C	4.9	2.2	143.15***
50° to 55°C	5.8	2.1	280.33***
55° to 60°C	7.0	2.2	151.66***
60° to 65°C	8.1	2.3	168.96***
65° to 70°C	8.9	2.9	175.63***
70° to 75°C	10.5	3.0	157.50***
75° to 80°C	13.3	3.1	64.59***

[a]Includes data for both end point temperatures

***$P \leq 0.001$

Fig. 6-Rate of heat penetration from initial temperature to 70° or 80°C for top round steaks cooked at 94° or 149°C.


END POINT TEMP. - 70 °C
MINUTES
80
70
60
50
40
30
20
10
0
10
20
30
40
50
60
70
TEMPERATURE, °C
OVEN TEMP.
94 °C
149 °C

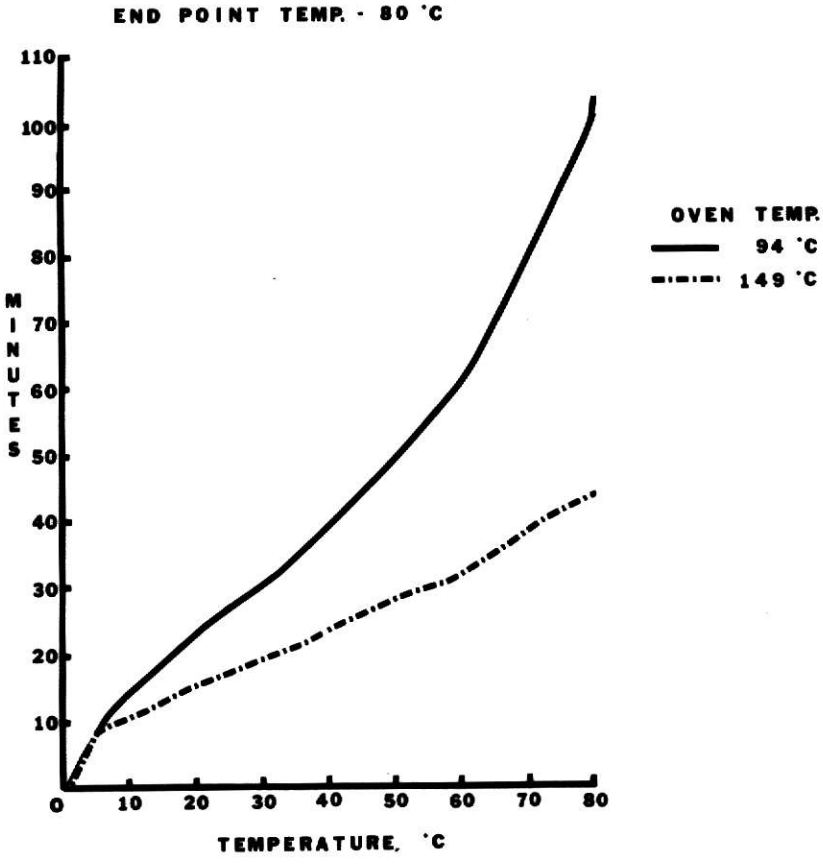

END POINT TEMP. - 80 °C
MINUTES
110
100
90
80
70
60
50
40
30
20
10
0
10
20
30
40
50
60
70
80
TEMPERATURE, °C
OVEN TEMP.
94 °C
149 °C

Fig. 7-Drip cooking losses from top round steaks cooked at 94°C to end point temperatures of 70° or 80°C.

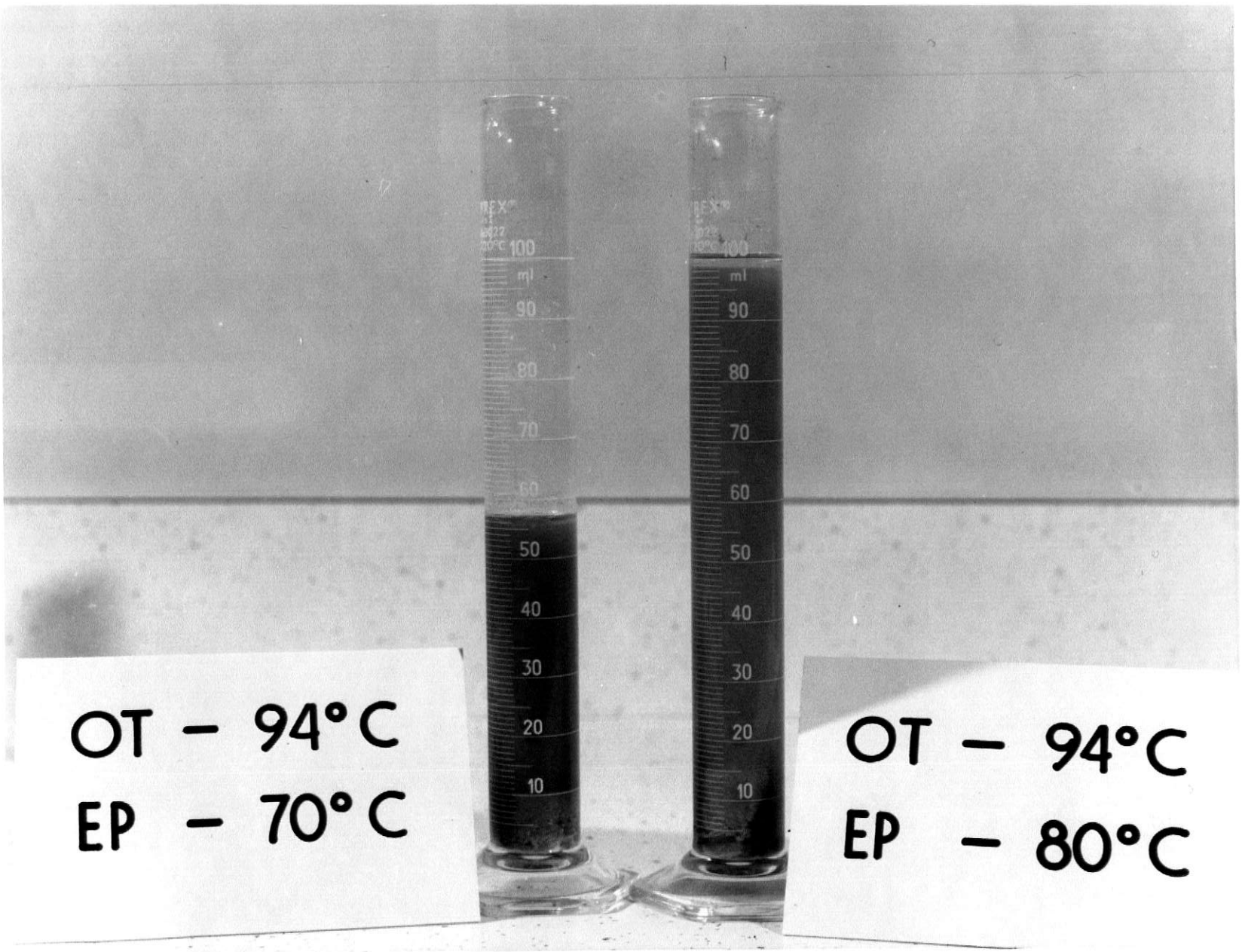
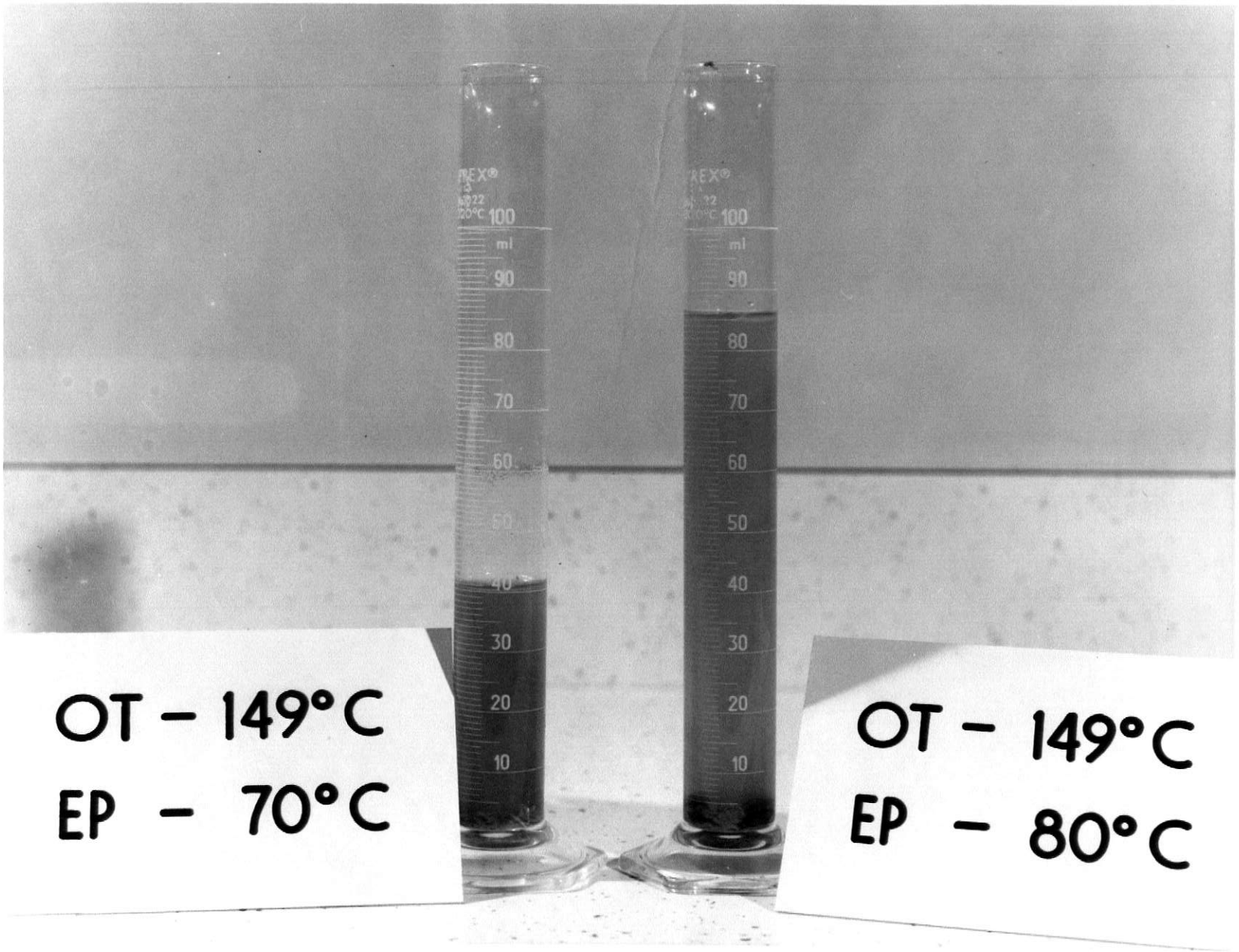

OT – 94°C
EP – 70°C
OT – 94°C
EP – 80°C

Fig. 8-Drip cooking losses from top round steaks cooked at 149°C to end point temperatures of 70° or 80°C.


OT - 149°C
EP - 70°C
OT - 149°C
EP - 80°C

Data in this study agree with those of Bayne et al. (1971) who oven roasted (dry heat) SM muscle at 93° or 149°C to an end point of 70°C. Cooking time was longer ($P \leq 0.01$) and total cooking losses higher ($P \leq 0.01$) for cuts roasted at 93°C than at 149°C. Percentage collagen solubilized was calculated using hydroxyproline content and a conversion factor of 13%. Similar to the results of this study, they found no significant difference between the two oven temperatures with respect to percentage collagen solubilized during heating.

End point temperature

Data for effects of end point temperature are presented in Table 3. As total cooking time ($P \leq 0.001$), total cooking losses ($P \leq 0.001$) and drip losses ($P \leq 0.01$) increased with an increased end point temperature, total moisture and sensory scores for tenderness and softness ($P \leq 0.01$) decreased. The increased amount of drip and slight increase in coagulum at 80°C can be observed in Figures 7 and 8.

Relationships between selected paired variates

Correlation coefficients were calculated to study relationships between selected measurements for each oven temperature and each end point temperature (Table 5). Relationships are discussed according to Shindell (1964) who considered a coefficient between 0.00 and 0.39, irrespective of sign, a low correlation; a coefficient between 0.40 and 0.79 was designated a moderate relationship; and one of 0.80 or above was considered a good relationship.

Moderate to approaching good correlations occurred for six of the eight paired variates studied when steaks were cooked at 94°C to 70°C. Hydroxyproline solubilized increased with total cooking time ($r = 0.79^*$), total cooking losses ($r = 0.66$) and drip cooking losses ($r = 0.67$). As time the internal temperature of steaks was in the 55° to 60°C interval increased, hydroxyproline solubilized increased ($r = 0.42$), tenderness score decreased ($r = -0.66$) and softness score decreased ($r = -0.61$).

Moderate to good relationships existed for all eight paired variates studied when steaks were cooked at 94°C to 80°C. Relationships between hydroxyproline solubilized, total cooking time and both total and drip

Table 5-Correlation coefficients for selected paired variates of cooked meat

Paired variates d/f = 6	Oven temperature, °C			
	94°		149°	
	End point temp., °C		End point temp., °C	
	70°	80°	70°	80°
Hydroxyproline solubilized, % vs:				
Total cooking time, min	0.79*	0.52	0.19	0.20
Total cooking losses, %	0.66	0.54	0.16	-0.09
Drip cooking losses, %	0.67	-0.53	0.42	0.22
Shear value, kg/1.3-cm core	0.10	-0.61	0.20	0.09
Tenderness score	0.34	0.77*	0.30	0.48
55° to 60°C interval, min vs:				
Hydroxyproline solubilized, %	0.42	0.70	0.09	-0.63
Tenderness score	-0.66	0.86**	0.09	-0.10
Softness score	-0.61	0.73	0.16	-0.06

d/f = 6; *P $\leq$ 0.05, r = 0.707; **P $\leq$ 0.01, r = 0.834

cooking losses were poorer for those paired variates when steaks were cooked at 94°C to 70°C. Relationships were better for hydroxyproline vs shear value (r = -0.61) and tenderness score (r = 0.77*), Table 5. As percentage hydroxyproline solubilized increased, shear values decreased. Other moderate to approaching good relationships associated with cooking at 94°C to 80°C were time internal temperature of steaks was between 55° and 60°C vs hydroxyproline solubilized (r = 0.70), tenderness score (r = 0.86**) and softness score (r = 0.73*).

Bayne et al. (1971) reported that the percentage collagen solubilized (as measured by hydroxyproline content) was not related to shear value, but was related to tenderness score (r = 0.657*). Paul et al. (1973) reported that correlation coefficients for percentage collagen solubilized vs shear values were 0.09 ns for SM and -0.45* for BF. Penfield and Meyer (1975) found a moderate correlation (r = -0.704**) for percentage hydroxyproline solubilized vs shear value. As percentage hydroxyproline solubilized increased, shear value decreased. In this study hydroxyproline solubilized was related poorly to both shear value and tenderness score when steaks were cooked at 94°C to 70°C, but when cooking was at 94°C to 80°C r values for shear value and tenderness score were -0.61 and 0.77*, respectively.

Bramblett et al. (1959) found that the amount of time the internal temperature was between 57° and 60°C was related to tenderness as shown by a negative correlation (r = -0.73***) for the length of time the meat was in this temperature range vs shear value. Vollmar et al. (1976) noted that for roasts cooked by moist heat as the length of time internal temperature was between 55° and 70°C increased, tenderness and mealiness scores decreased slightly, and shear values increased slightly.

With an oven temperature of 149°C and end points of 70°C or 80°C most of the correlation coefficients were lower than for their counterparts with an oven temperature of 94°C (Table 5). Hydroxyproline solubilized was related moderately only to drip cooking losses with an end point of 70°C (r = 0.42). Hydroxyproline solubilized was related moderately to tenderness score (r = 0.48) and to time internal temperature was in the 55° to 60°C interval when cooked to 80°C (r = -0.63). As time in the 55° to 60°C interval increased, hydroxyproline solubilized decreased.

Oven temperature x end point temperature interactions

Total cooking time and drip cooking losses were the only measurements for which there was a significant interaction between oven temperature and end point temperature. Data for those measurements are presented in Table 6. For both end point temperatures, total cooking time was longer ($P \leq 0.01$) for steaks cooked at 94°C than for those cooked at 149°C. At oven temperature 94°C, but not at 149°C, the time required to reach an internal temperature of 70°C was less ($P \leq 0.01$) than the time required to reach an internal temperature of 80°C. Drip cooking losses were greater ($P \leq 0.05$) for oven temperature 94°C, end point temperature 80°C than for the other three treatment combinations. Data for the other measurements on the basis of oven temperature and end point temperature are presented in Table 13, Appendix, p. 65.

Table 6-Means and F-values for significant oven temperature x end point interactions

Measurement	Oven temp., °C	End point temp., °C 70°	80°	F-value
Total cooking time, min	94°	78.8	103.3	12.94**
	149°	38.3[a]	44.3[a]	
Drip cooking losses, %	94°	27.7[b]	33.8	7.04*
	149°	25.9[b]	26.2[b]	

[a,b]Means for a measurement bearing the same letter do not differ significantly ($P \leq 0.05$)

*$P \leq 0.05$; **$P \leq 0.01$

Hydroxyproline solubilized

Hydroxyproline solubilized was not affected by oven or end point temperature. Percentage hydroxyproline solubilized among the four treatment combinations ranged from 1.47% to 1.80%.

Several variables should be considered when measuring hydroxyproline content. The effect of reagent concentration and stability and reaction time on color development was studied by Bergman and Loxley (1963). The variables studied included optimum time and temperature for oxidation and color development, as well as the optimum concentrations of organic solvent and of the reagents at each stage of the procedure. Also, the availability of hydroxyproline to combine with the Erlich's reagent should be considered. Verzar (1964) found that the quantity of hydroxyproline released during heating declined with increased maturity of the muscle. He attributed this decline to the increased cross-linking of collagen during aging. Woessner (1961) found hydroxyproline in the enzymes collagenase and elastase. He suggested that studies investigating hydroxyproline liberation should have adequate controls to avoid contributions of hydroxyproline from the enzymes.

Variation in values reported for hydroxyproline solubilized may result from differences in calculations. For example, percentage hydroxyproline solubilized ranged from 4.25% to 13.57% in a study reported by Paul et al. (1973) and from 1.95% to 13.43% in a study reported by Penfield and Meyer (1975). The calculations used in the study by Paul et al. (1973) were not given in the paper. Communications with one of the authors of the study reported by Penfield and Meyer (1975) revealed that they did not use hydroxyproline content of the raw sample in their calculations. In this study a raw sample was analyzed for total hydroxyproline content of the muscle and both cooked meat and drip cooking losses were analyzed for hydroxyproline solubilized during cooking. These values were used in calculating the percentage hydroxyproline solubilized (Appendix, p. 56, 57).

Differences between raw and cooked muscle

Data for selected measurements on raw and cooked muscle are presented in Table 7. As expected, percentage total moisture was less ($P \leq 0.001$) and percentage ether extract was greater ($P \leq 0.001$) in the cooked muscle than in the raw muscle. There was no significant difference in pH between cooked and raw muscle.

Table 7-Means and F-values for selected measurements on raw and cooked muscle [a]

Measurement	Raw Muscle	Cooked Muscle	Difference	F-value
Total moisture, %	73.3	61.0	12.3	335.76***
Ether extract, %	3.4	5.3	1.9	8.22***
pH	5.2	5.3	0.1	0.001

[a]Includes data for all four treatment combinations
***$P \leq 0.001$

SUMMARY

A randomized complete block design was followed to cook 32 top round steaks in oven film bags at 94° or 149°C to 70° or 80°C. Total cooking time ($P \leq 0.001$), drip cooking losses ($P \leq 0.001$) and softness scores ($P \leq 0.05$) were higher for steaks cooked at 94°C than for those cooked at 149°C. Measurements affected by end point temperature were total cooking time, total cooking losses and total moisture ($P \leq 0.001$) and drip cooking losses and sensory scores for tenderness and softness ($P \leq 0.01$). As total cooking time and total and drip cooking losses increased with an increased end point temperature, total moisture and sensory scores for tenderness and softness decreased. The rate at which heat penetrated the muscle was greater ($P \leq 0.001$) for steaks cooked at 149°C than for those cooked at 94°C. At oven temperature 94°C, end point temperature 80°C percentage hydroxyproline solubilized was correlated moderately with tenderness score ($r = 0.73^*$) and tenderness was highly correlated with shear value ($r = 0.83^{**}$) and with length of time the steak was held at an internal temperature of 55° to 60°C ($r = 0.86^{**}$). Values for total moisture were less ($P \leq 0.001$) and values for ether extract were greater ($P \leq 0.001$) in cooked than in raw muscle. There were no significant differences between oven temperatures or between end point temperatures for percentage hydroxyproline solubilized, shear value, ether extract or pH.

CONCLUSIONS

Under the conditions of this study it was concluded that:

1. Oven temperatures of 94° or 149°C, and end point temperatures of 70° or 80°C do not differ in their effect on collagenous tissue in bovine top round steaks. Percentage hydroxyproline solubilized is not significantly different for the two oven temperature or between the two end point temperatures.
2. Hydroxyproline values are related moderately to tenderness scores and shear values with an oven temperature of 94°C and an end point of 80°C.
3. Percentage hydroxyproline solubilized and tenderness and softness scores are related moderately to the time internal temperature of the steaks is between 55° and 60°C at an oven temperature of 94°C.

REFERENCES

Adams, R., Harrison, D. L. and Hall, J. L. 1960. Comparison of enzyme and Waring Blendor methods for the determination of collagen in beef. J. Agr. Food Chem. 8: 229.

AOAC. 1976. "Official Methods of Analysis," 12th ed. p. 417. Assoc. of Official Analytical Chemists, Washington, D. C.

Bayne, B. H., Strawn, S. S., Hutton, C. W., Backus, W. R. and Meyer, B. H. 1971. Relation of alkali and ringer insoluble collagen to tenderness of beef heated at two rates. J. Animal Sci. 33: 958.

Bayne, B. H., Allen, M. B., Large, N. F., Meyer, B. H. and Goertz, G. E. 1973. Sensory and histological characteristics of beef rib cuts heated at two rates to three end point temperatures. Home Econ. Research J. 2: 29.

Bergman, I. and Loxley, R. 1963. Two improved and simplified methods for colorimetric determination of hydroxyproline. Anal. Chem. 35: 1961.

Birkner, M. L. and Auerbach, E. 1960. Microscopic structure of animal tissue. In "The Science of Meat and Meat Products," p. 10. W. H. Freeman and Co., San Francisco, California.

Blackburn, S. 1968. Spectrophotometric methods of estimation. In "Amino Acid Determination," p. 195. Marcel Dekker, Inc., New York.

Blumenkrantz, N. and Asboe-Hansen, G. 1975. Assay for hydroxyproline and proline on one sample and a simplified method for hydroxyproline. Anal. Biochem. 63: 331.

Bramblett, V. D., Hostetler, R. L., Vail, G. E. and Draudt, H. N. 1959. Qualities of beef as affected by cooking at very low temperatures for long periods of time. Food technol. 13: 707.

Bramblett, V. D. and Vail, G. E. 1964. Further studies on the qualities of beef as affected by cooking at very low temperatures for long periods of time. Food Technol. 18: 245.

Cassens, R. G. 1971. Microscopic structure of animal tissues. In "The Science of Meat and Meat Products," p.11. W. H. Freeman and Co., San Francisco, California.

Cover, S. 1941. Comparative cooking time and tenderness of meat cooked in water and in an oven of the same temperature. J. Home Econ. 33: 596.

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

Cover, S. and Smith, W. H. Jr. 1956. The effect of two methods of cooking on palatability scores, shear force values, and collagen content of two cuts of beef. Food Research 21: 312.

Cover, S., Bannister, J. A. and Kehlenbrink, E. 1957. Effect of four conditions of cooking on the eating quality of two cuts of beef. J. Food Sci. 22: 635.

Cover, S., Ritchey, S. J. and Hostetler, R. L. 1962a. Tenderness of beef. I. The connective tissue component of tenderness. J. Food Sci. 27: 469.

Cover, S., Ritchey, S. J. and Hostetler, R. L. 1962b. Tenderness of beef. III. The muscle-fiber components of tenderness. J. Food Sci. 27: 483.

Cross, H. R., Carpenter, Z. L. and Smith, G. C. 1973. Effects of intramuscular collagen and elastin on bovine muscle tenderness. J. Food Sci. 38: 998.

Ferger, D. C., Harrison, D. L. and Anderson, L. L. 1972. Lamb and beef roasts cooked from the frozen state by dry and moist heat. J. Food Sci. 37: 226.

Field, R. A., Pearson, A. M. and Schweigert, B. S. 1970a. Labile collagen from epimysial and intramuscular connective tissue as related to Warner-Bratzler shear values. J. Agr. Food Chem. 18: 280.

Field, R. A., Pearson, A. M. and Schweigert, B. S. 1970b. Hydrothermal shrinkage of bovine collagen. J. Animal Sci. 30: 712.

Fukuzawa, T. and Briskey, E. J. 1970. Morphological and biophysical properties of the myofibril. In "The Physiology and Biochemistry of Muscle as a Food, 2." p. 395. The University of Wisconsin Press, Madison, Wisconsin.

Goll, D. E., Bray, R. W. and Hoekstra, W. G. 1963. Age-associated changes in muscle composition. The isolation and properties of a collagenous residue from bovine muscle. J. Food Sci. 28: 503.

Goll, D. E., Hoekstra, W. G. and Bray, P. W. 1964. Age associated changes in bovine muscle connective tissue. II. Exposure to increasing temperature. J. Food Sci. 29: 615.

Griswold, R. M. 1955. The effect of different methods of cooking beef round of commercial and prime grades. II. Collagen, fat and nitrogen content. Food Research 20: 171.

Hall, D. A. 1961. "The Chemistry of Connective Tissue," pp. 25-52. Charles Thomas, Springfield, Illinois.

Harrison, D. L., Vail, G. E., Hall, J. L. and Mackintosh, D. L. 1953. Household cooking methods for commercial grade beef. Unpublished manuscript, Kansas State University, Manhattan, Kansas.

Harrison, D. L. 1975. Effects of heat treatments on bovine skeletal muscle. Texas Nutrition Conference Proceedings.

Herring, H. K., Cassens, R. G. and Briskey, E. J. 1967. Factors affecting collagen solubility in bovine muscles. J. Food Sci. 32: 534.

Hiner, R. L., Anderson, E. E. and Fellers, C. R. 1955. Amount and character of connective tissue as it relates to tenderness in beef muscle. Food Technol. 9: 80.

Hood, M. P. 1960. Effect of cooking method and grade on beef roasts. J. Am. Dietet. Assoc. 37: 363.

Hostetler, R. L. and Landmann, W. A. 1968. Photomicrographic studies of dynamic changes in muscle fiber fragments. 1. Effect of various heat treatments on length, width and birefringence. J. Food Sci. 33: 468.

Husaini, S. A., Deatherage, F. E., Kunkle, L. E. and Draudt, H. N. 1950. Studies on meat I. The biochemistry of beef as related to tenderness. Food Technol. 4: 313.

Hutterer, F. and Singer, E. J. 1960. A modified method for hydroxyproline determination. Anal. Chem. 32: 556.

Irwin, I. and Cover, S. 1959. Effect of dry heat method of cooking on collagen content of two beef muscles. Food Technol. 13: 655.

Kim, C. W., Ho, G. P., and Ritchey, S. J. 1967. Collagen content and subjective scores for tenderness of connective tissue in animals of different ages. J. Food Sci. 32: 586-588.

Kivirikko, K. I., Laitinen, O. and Prockop, J. 1967. Anal. Biochem. 19: 249.

Laakkonen, E., Wellington, G. H. and Sherbon, J. W. 1970a. Low-temperature, long-time heating of bovine muscle. 1. Changes in tenderness, water-holding capacity, pH and amount of water soluble components. J. Food Sci. 35: 175.

Laakkonen, E., Sherbon, J. W. and Wellington, G. H. 1970b. Low-temperature, long-time heating of bovine muscle. 3. Collagenolytic activity. J. Food Sci. 35: 181.

Lawrie, R. A. 1968. Chemical changes in meat due to processing--a review. J. Sci. Food Agr. 19: 233.

Loyd, E. J. and Hiner, R. L. 1959. Meat tenderness - relation between hydroxyproline of alkali-insoluble protein and tenderness of bovine muscle. J. Agr. Food Chem. 7: 860.

McClain, P. E., Mullins, A. M., Hansard, S. L., Fox, J. D. and Boulware R. F. 1965. Relationship of alkali insoluble collagen to tenderness of three bovine muscles. J. Animal Sci. 24: 1107.

McCrae, S. E. and Paul, P. C. 1974. Rate of heating as it affects the solubilization of beef muscle collagen. J. Food Sci. 39: 18.

Machlik, S. M. and Draudt, H. N. 1963. The effect of heating time and temperature on the shear of beef semitendinosus muscle. J. Food Sci. 28: 711.

Meyer, L. H. 1968. "Food Chemistry." pp. 174-180, Reinhold Publishing, N. Y.

Miller, M. and Kastelic, J. 1956. Chemical responses of connective tissue of bovine skeletal muscle. J. Agr. Food Chem. 4: 537.

Neuman, R. E. and Logan, M. A. 1950. The determination of hydroxyproline. J. Biol. Chem. 184: 299.

Parrish, F. C., Bailey, M. E. and Naumann, H. D. 1962. Hydroxyproline as a measure of beef tenderness. Food Technol. 16: 68.

Paul, P. C. 1957. Tenderness of Beef. J. Am. Dietet. Assoc. 33: 890-894.

Paul, P. 1963. Influence of methods of cooking on meat tenderness. In "Proceedings Meat Tenderness Symposium," P. 225. Campbell Soup Co., Camden, N. J.

Paul, P. C. 1972. Meat. In "Food Theory and Application," p. 335. John Wiley & Sons, Inc., New York, New York.

Paul, P. C. McCrae, S. E. and Hoffere, L. M. 1973. Heat-induced changes in extractability of beef muscle collagen. J. Food Sci. 38: 66.

Penfield, M. P. and Meyer, B. H. 1975. Changes in tenderness and collagen of beef semitendinosus muscle heated at two rates. J. Food Sci. 40: 150.

Prockop, J. and Udenfriend, S. 1960. A specific method for the analysis of hydroxyproline in tissues and urine. Anal. Biochem. 1: 228.

Reid, H. C. and Harrison, D. L. 1971. Effects of dry and moist heat on selected histological characteristics of beef semimembranosus muscle. J. Food Sci. 36: 206.

Ritchey, S. J. and Cover, S. 1962. Meat tenderness factors--Determination of collagen in raw and cooked beef from two muscles by alkali-insoluble, autoclave-soluble nitrogen and hydroxyproline content. J. Agr. Food Chem. 10: 40.

Ritchey, S. J., Cover, S. and Hostetler, R. L. 1963. Collagen content and its relation to tenderness of connective tissue in two beef muscles. Food Technol. 17: 76.

Satorius, M. J. and Child, A. M. 1938. Effect of coagulation on press fluid, shear force, muscle cell diameter and composition of beef muscle. Food Research 3: 619.

Shaffer, T. A., Harrison, D. L. and Anderson, L. L. 1973. Effects of end point and oven temperatures on beef roasts cooked in oven film bags and open pans. J. Food Sci. 38: 1205.

Shindell, S. 1964. "Statistics, Science, and Sense." University of Pittsburgh Press, Pittsburgh, Pennsylvania.

Shock, D. R., Harrison, D. L. and Anderson, L. L. 1970. Effects of dry and moist heat treatments on selected beef quality factors. J. Food Sci. 35: 195.

Skelton, M., Harrison, D. L., Hall, J. L., Adams, R. and Goertz, G. E. 1963. Effect of degree of doneness on collagen nitrogen and collagenous tissue in two muscles of beef. Food Technol. 17: 93.

Stegemann, H. and Stalder, K. 1967. Determination of hydroxyproline. Clin. Chim. Acta. 18: 267.

Troll, W. and Cannan, R. K. 1953. A modified photometric ninhydrin method for the analysis of amino and imino acids. J. Biol. Chem. 200: 803.

Venable, J. H. 1963. The histology of muscle. In "Proceedings Meat Tenderness Symposium," p. 7. Campbell Soup Co., Camden, New Jersey.

Verzar, F. 1963. Aging of collagen fiber. In "International Review of Connective Tissue Research," Vol. 2, p. 243. Academic Press, New York.

Vollmar, E. K., Harrison, D. L. and Hogg, M. G. 1976. Bovine muscle cooked from the frozen state at low temperature. J. Food Sci. 41: 411.

Vognarova, I., Dvorak, Z. and Bohn, R. 1968. Collagen and elastin in different cuts of veal and beef. J. Food Sci. 33: 339.

Weir, C. E. 1960. Palatability characteristics of meat. In "The Science of Meat and Meat Products," p. 212. W. H. Freeman and Co., San Francisco, California.

Weirbicki, E. and Deatherage, F. E. 1954. Hydroxyproline as an index of connective tissue in muscle. J. Agr. Food Chem. 2: 878.

Winegarden, M. W., Lowe, B., Kastelic, J., Kline, E. A., Plagge, A. R. and Shearer, P. S. 1952. Physical changes of connective tissue of beef during heating. Food Research 17: 172.

Woessner, J. F., Jr. 1961. The determination of hydroxyproline in tissue and protein samples containing small portions of this imino acid. Arch. Biochem. and Biophysics. 93: 440.

Yang, S. P. and Couvillion, L. A. 1964. Collagen content of two beef muscles. J. Animal Sci. 23: 865.

ACKNOWLEDGMENTS

The author wishes to express sincere appreciation to Dr. Dorothy L. Harrison, Professor of Foods and Nutrition and Major Professor, for suggesting the problem, for assistance and guidance in preparing the manuscript and for encouragement throughout the period of graduate study. The writer wishes to thank Dr. Arthur D. Dayton, Associate Professor and Head Department of Statistics, for analysis of the data; Dr. Jane A. Bowers, Professor and Head Department of Foods and Nutrition, and Dr. Willard S. Ruliffson, Professor of Biochemistry, for serving on the writer's committee; Mrs. Karen Clithero Dudte, Nesa Leung, and Mrs. Kathy Ottoway for laboratory assistance; and members of the sensory panel for evaluating the samples.

The author wishes to thank her special friends and fellow students for their support. Sincere appreciation is expressed to my mother and my fiance for their inspiration and encouragement during the period of my graduate study.

APPENDIX

Procedure for Spectrophotometric Determination of Hydroxyproline Content of Bovine Muscle (Bergman and Loxley, 1963; Penfield and Meyer, 1975; Stegemann and Stalder, 1967; and Woessner, 1961)

Preparation of raw sample

1. Weigh 1g ground meat sample and homogenize with 30 ml distilled, deionized water (40°C) for 4 minutes at high speed in a Waring blendor.

2. Pour homogenate into a 100 ml volumetric flask and add 50 ml concentrated HCl. Fill to volume with distilled, deionized water.

3. Place 25 ml aliquots of the homogenate into each of two 50 ml ampoules. Use a blow torch to seal top of ampoules.

4. Hydrolyze samples in 107°C oven for 16 hours.

5. Break tops off ampoules and pour contents of each ampoule into a 500 ml Erlenmeyer flask.

6. Add 4 drops of 0.02% methyl red indicator to the flask. Adjust pH to 6 or 7 using approximately 125 ml of 2.5 N NaOH. (Prepare 2.5 N NaOH by dissolving 100 g NaOH pellets in 1 liter distilled water. In this study 100 to 150 ml of NaOH were required with an estimated mean amount of 125 ml NaOH. This estimated quantity plus the two 25 ml aliquots of the homogenate give a total of 175 ml solution.)

7. Use a Buchner funnel for vacuum filtration of the solution.

Preparation of cooked samples and drippings

1. Weigh 5 g of cooked, ground meat or 5 g drippings.

2. Homogenize in a Waring blendor with 30 ml of distilled, deionized water (40°C) for 3 minutes.

3. Centrifuge at 4,600 X G for 15 minutes.

4. Decant supernatant through cheesecloth into a 100 ml volumetric flask. Make up to volume with distilled, deionized water.

Preparation of standards

1. Weigh 25 mg (0.025 g) L-hydroxyproline. Dissolve in 250 ml of 0.001 N HCl. This solution can be stored in a glass stoppered bottle in a refrigerator (4°C) indefinitely.

2. Label six volumetric flasks (100 ml) with a number (0 - 5). Prepare standards daily by using following dilutions.

Flask No.	Concentration	ml Stock Solution	ml Distilled Water
0	0 mcg/ml	0.0	100.0
1	0.5 mcg/ml	0.5	99.5
2	1.0 mcg/ml	1.0	99.0
3	1.5 mcg/ml	1.5	98.5
4	2.0 mcg/ml	2.0	98.0
5	2.5 mcg/ml	2.5	97.5

Preparation of reagent solutions

Buffer solution

1. Weigh the following: 57.0 g sodium acetate trihydrate
   37.5 g sodium citrate ($2H_2O$)
   5.5 g citric acid (H_2O)

2. Measure 385 ml isopropanol

3. Place chemicals in a 1000 ml volumetric flask and make up to volume with distilled, deionized water. This solution is stable indefinitely.

Oxidant solution

1. Prepare a 7% weight/volume aqueous solution of Chloramine T (the sodium salt of p-toluene sulfon-chloramide) allowing about 0.25 ml per analytical determination.

2. Just before the start of each series of determinations, mix the Chloramine T solution with the buffer solution in the proportion of 1 volume Chloramine T to 4 volumes buffer to give the oxidant solution. (About 1.25 ml per analytical value is required).

Erlich's Reagent Solution

1. Dissolve p-Dimethylamino-benzaldehyde (p-dmab) in 60% perchloric acid in the proportions of 2 g of aldehyde to 3 ml of acid. (About 3 ml per analytical value is required).

2. Just before the start of a series of determinations, mix the above solution with isopropanol in the proportion of 3 volumes of the above solution to 13 volumes of isopropanol to give a final volume of about 16 ml per analytical value required, allowing for manipulation.

Procedure for hydroxyproline assay

1. Use clean dry Bausch and Lomb Spectronic 20 3/4" test tubes. Using duplicates, label 12 tubes (1 - 12) for hydroxyproline standards. Label the other tubes according to code decided upon for samples.

2. Pipet 1 ml portions of the hydroxyproline standard solutions or samples to be analyzed into the test tubes.

3. To each test tube add 2 ml of isopropanol and mix.

4. To each test tube add 1 ml of the oxidant solution from a pipet, mix and allow to stand 4 ($\pm$ 1) minutes.

5. To each test tube add 13 ml of the Erlich's reagent solution and mix well. Cover tubes with parafilm.

6. Heat tubes for 25 minutes at 60°C in a water bath. Cool tubes for 2 to 3 minutes in running tap water.

7. Measure absorbance immediately using a Bausch and Lomb Spectronic 20 at a wavelength of 558.

Preparation of standard curve

1. Subtract absorbance of the blank (average of tubes 1 and 2) from the absorbancies of the rest of the tubes.

2. Plot absorbancy vs mcg of hydroxyproline.

Calculations

1. Determine hydroxyproline content of samples by plotting absorbancy and reading mcg of hydroxyproline.

2. For raw strip samples:

$$\frac{0.5 \text{ g raw strip sample}}{175 \text{ ml}} = \frac{5 \text{ mg raw}}{175 \text{ ml}} = \frac{2.857 \text{ mg raw}}{1 \text{ ml}}$$

$$\frac{\dfrac{\text{mcg hydroxyproline in raw}}{1 \text{ ml sample}}}{2.857 \dfrac{\text{mg raw}}{1 \text{ ml}} \times \dfrac{1000 \text{ mcg}}{1 \text{ mg}}} \times 100 = \% \text{ hydroxyproline in raw strip sample}$$

% hydroxyproline in raw strip sample X g weight of raw steak = g hydroxyproline in raw steak

3. For cooked samples and drippings:

$$\frac{5 \text{ g meat (or drip)}}{100 \text{ ml}} = \frac{50 \text{ mg meat (or drip)}}{1 \text{ ml}}$$

$$\frac{\dfrac{\text{mcg hydroxyproline in meat sample (or drip sample)}}{1 \text{ ml sample}}}{\dfrac{50 \text{ mg meat (or drip)}}{1 \text{ ml}} \times \dfrac{1000 \text{ mcg}}{1 \text{ mg}}} \times 100 = \% \text{ hydroxyproline in cooked meat (or drip)}$$

% hydroxyproline in cooked steak X g weight of cooked steak = g hydroxyproline in cooked steak

% hydroxyproline in drip X g weight of drip = g hydroxyproline in drip

4. % hydroxyproline solubilized during cooking

$$\frac{\text{g hydroxyproline in drip} + \text{g hydroxyproline in cooked steak}}{\text{g hydroxyproline in raw steak}} \times 100 = \% \text{ hydroxyproline solubilized}$$

REFERENCES

Bergman, I. and Loxley, R. 1963. Two improved and simplified methods for colorimetric determination of hydroxyproline. Anal. Chem. 35: 1961.

Penfield, M. P. and Meyer, B. H. 1975. Changes in tenderness and collagen of beef semitendinosus muscle heated at two rates. J. Food Sci. 40: 150.

Stegemann, H. and Stalder, K. 1967. Determination of hydroxyproline. Clin. Chim. Acta. 18: 267.

Woessner, J. F., Jr. 1961. The determination of hydroxyproline in tissue and protein samples containing small portions of this imino acid. Arch. Biochem. and Biophysics. 93: 440.

Form I. Score Card for the Sensory Evaluation of Beef Top Round Steaks (Semimembranosus and Adductor Muscles).

Panel Member _____ Code _____ Date _____

Sample no.	Tenderness		Texture	
	Chews	Score[a]	Softness[b]	Mealiness[c]
1				
2				
3				
4				

Descriptive terms for scoring

[a]Tenderness

7 Extremely tender
6 Tender
5 Slightly tender
4 Neither tender nor tough
3 Slightly tough
2 Tough
1 Extremely tough

[b]Softness

7 Extremely soft
6 Soft
5 Slightly soft
4 Firm - neither soft nor hard
3 Slightly hard
2 Hard
1 Extremely hard

[c]Mealiness

7 Extremely mealy
6 Mealy
5 Slightly mealy
4 Neither mealy nor chewy
3 Slightly chewy
2 Chewy
1 Extremely chewy

Form II. Instructions to Judges for Sensory Evaluation of Beef Top Round (Semimembranosus and Adductor Muscles).

For scoring sensory evaluation, each judge is to select two cubes of meat from each double boiler. Use one cube for counting the number of chews and the tenderness score and the other for scoring the texture components.

Scoring for tenderness

Count the number of chews on a 1.3-cm cube of meat before swallowing. Chew until the cube is masticated completely, then swallow. Record a score of 7 to 1 that describes your impression of the tenderness of the cube. Refer to the score card for descriptive terms of specific scores within the range 7 to 1.

Use the number of chews to help you standardize your tenderness scores from day to day. Set up for yourself a range of the number of chews for each score from 7 to 1. For example, if you chew from 15 to 25 times, you might record a score of 7; if you chew 25 to 30 times, a score of 6; 35 to 45, a score of 5; continuing to reduce the score by a given number of increased chews. Each judge sets his own range of chews for a given score.

Scoring for texture

Texture is broken down into two components; softness and mealiness. Softness to tongue and cheek, and softness to tooth pressure (the muscular force exerted on the meat cube) should be considered when scoring for softness. Record a score for each sample within a range of 7 to 1, as indicated on the score card. Mealiness can be thought of as fragmentation of the meat resulting in tiny, dry and hard pieces of meat that cling to the cheek, gums, and tongue. Record a score for mealiness within a range of 7 to 1 that describes your impression of the sample. Refer to the score card for descriptive terms corresponding to each numerical score.

Comments

Comments about the sample and/or an explanation of why you gave a particular score to the sample are helpful.

Take your time to score each sample. Water is provided for rinsing your mouth between samples.

Table 8 -Means for significant differences attributable to weight of steak (replication)

Measurement	Replication 1	2	3	4	5	6	7	8
Initial weight, g	372.8[a]	341.3	347.3[a]	277.5[b]	327.3	287.3[c]	291.5[c]	280.8[b]
Total cooking time, min	68.3	75.0	70.8	62.5	67.0	65.0	61.8	58.8
Cooking losses, %								
Total	30.0	34.8	33.6	31.1	30.2	34.5	31.0	31.1
Drip	26.8	29.2	29.9	28.5	28.5	29.4	26.9	27.7
Total moisture, %	61.2	58.8	60.3	61.0	62.2	61.4	60.1	62.6
Ether extract, %	5.3	5.5	6.8	6.6	4.2	4.7	5.2	4.6
pH	5.2[d]	5.3[e]	5.2[d]	5.2	5.3[e]	5.2	5.3[e]	5.3
Shear value, kg/1.3-cm core	2.7	3.6	3.2	3.0	3.2	3.4	2.6	2.4
Hydroxyproline solubilized, %	2.2	1.9	1.5	1.8	1.8	1.6	1.1	1.1
Sensory scores[f]								
Tenderness	5.3	5.1	4.5	5.2	4.6	4.6	4.5	4.6
Softness	4.9	4.6	4.2	4.2	4.1	4.0	4.3	4.1
Mealiness	4.8	4.7	4.4	4.2	4.5	4.3	4.7	4.0

[a,b,c,d,e]Means bearing same letters do not differ significantly ($P \leq 0.05$)

[f]7-(extremely tender, extremely soft, extremely mealy);
1-(extremely tough, extremely hard, extremely chewy)

Table 9-Rate of heat penetration, in minutes, in individual steaks to increase by increments of 5°C from initial temperature to 70°C.

Oven temperature, 94°C

End point temp.	Initial temp.	5°	10°	15°	20°	25°	30°	35°
70°C	4	3	8	14	19	23	27	32
	-2	25	29	33	37	41	44	47
	0	9	14	21	26	29	35	40
	3	6	10	14	18	22	26	30
	6	-	10	14	18	23	27	32
	2	6	13	19	25	30	36	41
	6	-	7	11	15	19	24	29
	6.5	-	8	12	15	18	22	29
Mean	3.2	6.1	12.4	17.3	21.6	25.6	30.1	34.5

	40°	45°	50°	55°	60°	65°	70°
	38	43	49	54	60	69	79
	51	55	59	64	69	75	82
	46	52	57	65	74	83	93
	35	40	45	51	57	64	73
	36	41	46	51	59	67	76
	47	51	55	60	68	77	86
	34	39	44	50	56	62	73
	29	33	38	43	50	59	68
Mean	39.5	44.3	49.1	54.8	61.6	69.5	78.8

Table 10-Rate of heat penetration, in minutes, in individual steaks to increase by increments of 5°C from initial temperature to 80°C.

Oven temperature, 94°C

End point temp., °C	Initial temp., °C	5°	10°	15°	20°	25°	30°	35°
80	-1	11	16	22	28	32	36	41
	-2	16	21	26	31	36	40	46
	-2	17	22	27	31	35	39	43
	-1	11	14	17	20	24	27	31
	4	3	11	17	23	28	34	39
	1	7	13	17	22	25	29	33
	8	-	5	10	14	17	20	23
	6	-	11	15	19	22	25	28
Mean	1.6	8.1	14.1	18.9	23.5	27.4	31.3	35.5

	40°	45°	50°	55°	60°	65°	70°	75°	80°
	46	51	56	62	70	79	86	98	111
	51	56	60	65	74	85	93	105	122
	48	53	57	63	69	77	85	95	106
	35	40	44	50	57	63	70	80	89
	44	50	56	61	69	75	89	100	116
	37	41	46	53	58	67	75	82	90
	26	31	38	45	53	63	73	83	96
	31	35	40	45	51	58	65	77	96
Mean	39.8	44.6	49.6	55.5	62.6	70.9	79.5	90	103.3

Table 11-Rate of heat penetration, in minutes, in individual steaks to increase by increments of 5°C from initial temperature to 70 °C.

Oven temperature, 149°C

End point temp.	Initial temp.	5°	10°	15°	20°	25°	30°	35°
70°C	-0.5	7	10	12	14	16	18	20
	-2	18	21	23	25	27	30	32
	2	3	7	9	12	14	16	18
	-2	16	19	21	23	25	27	29
	2	4	7	10	13	15	17	19
	0	13	16	18	20	22	24	26
	1	6	9	11	13	15	17	19
	2	4	7	10	12	14	16	18
Mean	0.3	9.6	12.0	14.3	16.5	18.5	20.6	22.6

40°	45°	50°	55°	60°	65°	70°
22	24	26	28	30	32	35
34	36	38	40	42	44	46
21	23	25	27	29	31	34
31	33	35	37	39	41	44
21	23	26	28	30	32	35
28	31	33	35	38	41	44
21	23	25	27	29	31	34
20	22	24	26	28	31	34
24.8	26.9	29.0	31.0	33.1	35.4	38.3

Mean

Table 12-Rate of heat penetration, in minutes, in individual steaks to increase by increments of 5°C from initial temperature to 80°C.

Oven temperature, 149°C

End point temp.	Initial temp.	5°	10°	15°	20°	25°	30°	35°
80°C	-2	14	17	19	21	23	25	27
	-2	14	16	18	20	22	24	26
	0	7	11	14	16	19	22	25
	1	7	10	12	14	16	18	20
	1	7	10	12	14	16	18	20
	-1	8	10	12	14	16	18	20
	0.5	7	10	12	14	16	18	21
	4	2	5	8	10	12	14	16
Mean	0.9	8.3	11.1	13.4	15.4	17.5	19.6	21.9

	40°	45°	50°	55°	60°	65°	70°	75°	80°
	29	31	34	36	38	40	43	46	48
	28	30	32	34	36	40	43	46	50
	27	29	32	35	38	41	44	47	50
	23	26	28	30	32	34	37	40	44
	22	24	26	28	30	32	35	38	41
	22	24	26	28	30	32	35	38	40
	23	25	27	30	33	35	38	41	44
	18	20	22	24	26	28	30	33	37
Mean	24.0	26.1	28.3	30.6	32.9	35.3	38.1	41.1	44.3

Table 13-Means and F-values for 10 measurements by oven temperature and end point temperature.

Measurement	Oven temp., °C	End point temp., °C 70°	80°	F-value
Initial weight, g	94°	322.9	310.4	1.95
	149°	302.6	326.9	
Total cooking losses, %	94°	28.6	35.7	4.08
	149°	30.5	33.5	
Total moisture, %	94°	62.7	59.3	0.67
	149°	62.1	59.7	
Ether extract, %	94°	5.0	5.6	0.55
	149°	4.7	6.1	
pH	94°	5.3	5.2	0.67
	149°	5.3	5.2	
Shear value, g/1.3-cm core	94°	2.8	3.0	2.13
	149°	3.4	3.0	
Hydroxyproline solubilized, %	94°	1.5	1.8	0.04
	149°	1.5	1.7	
Sensory scores[a]				
Tenderness	94°	5.2	4.6	1.55
	149°	4.9	4.6	

Table 13-(concluded)

Measurement	Oven temp., °C	End point temp., °C		F-value
		70°	80°	
Softness	94°	5.2	4.0	3.32
	149°	4.1	3.9	
Mealiness	94°	4.5	4.7	0.23
	149°	4.1	4.5	

[a]7-(extremely tender, extremely soft, extremely mealy);
1-(extremely tough, extremely hard, extremely chewy)

Table 14- Initial weight of and total cooking time for individual steaks

Measurement		Oven temperature, °C: 94°, End point temp, °C: 70°	94°, End point temp, °C: 80°	149°, End point temp., °C: 70°	149°, End point temp., °C: 80°
Initial weight, g		376	384	349	382
		332	366	365	302
		403	324	286	376
		253	206	335	316
		319	365	295	330
		310	285	241	313
		315	290	291	270
		275	263	259	326
	Mean	323	310	303	327
Total cooking time, min		79	111	35	48
		82	122	46	50
		93	106	34	50
		73	89	44	44
		76	116	35	41
		86	90	44	40
		73	96	34	44
		68	96	34	37
	Mean	79	103	38	44

Table 15 -Percentage total and drip cooking losses for individual steaks

Measurement		Oven temperature, °C			
		94°		149°	
		End point temp., °C		End point temp., °C	
		70°	80°	70°	80°
Total cooking losses, %		27.0	36.0	26.0	31.0
		27.4	36.6	36.	38.7
		30.3	35.5	33.2	35.4
		25.3	35.4	28.4	35.4
		26.3	35.1	27.5	32.1
		34.8	34.7	37.8	31.0
		27.0	36.2	26.8	34.1
		30.5	36.2	27.8	30.1
	Mean	28.6	35.7	30.5	33.5
Drip cooking losses, %		26.0	34.0	22.0	25.0
		27.1	30.1	31.2	28.4
		29.5	34.0	27.6	28.7
		24.5	35.0	26.3	28.5
		25.1	34.0	25.8	29.4
		33.5	34.0	29.5	20.8
		26.7	34.5	24.1	22.6
		29.1	34.6	20.8	26.4
	Mean	27.7	33.8	25.9	26.2

Table 16-Percentage total moisture, ether extract and pH for individual steaks

Measurement		Oven temperature, °C			
		94°		149°	
		End point temp., °C		End point temp., °C	
		70°	80°	70°	80°
Total moisture, %		63.0	57.7	63.1	61.1
		61.8	57.5	60.2	55.9
		60.4	60.4	60.3	60.0
		66.3	59.7	62.1	56.0
		64.3	60.2	63.2	61.2
		61.4	60.1	62.6	61.7
		62.4	58.6	62.2	57.3
		62.4	60.0	63.4	64.5
	Mean	62.7	59.3	62.1	59.7
Ether extract, %		4.3	6.3	3.7	7.1
		5.4	6.5	3.9	6.2
		8.3	6.2	8.2	4.7
		3.3	6.3	6.2	10.6
		4.0	4.2	4.0	4.5
		6.0	4.0	4.8	4.0
		4.5	5.8	4.1	6.4
		4.2	5.4	3.0	5.7
	Mean	5.0	5.6	4.7	6.1

Table 16-(concluded)

Measurement	Oven temperature, °C			
	94°		149°	
	End point temp., °C		End point temp., °C	
	70°	80°	70°	80°
pH	5.3	5.3	5.2	5.2
	5.4	5.2	5.3	5.3
	5.2	5.3	5.2	5.3
	5.2	5.1	5.2	5.2
	5.2	5.3	5.3	5.3
	5.3	5.3	5.3	5.2
	5.3	5.2	5.3	5.2
	5.3	5.4	5.3	5.3
Mean	5.3	5.2	5.3	5.2

Table 17-Warner-Bratzler shear values and percentage hydroxyproline solubilized for individual steaks

Measurement		Oven temperature, °C			
		94°		149°	
		End point temp., °C		End point temp., °C	
		70°	80°	70°	80°
Shear value, kg/1.3-cm core		3.1	2.1	2.7	3.1
		3.9	2.8	4.4	3.3
		2.4	4.0	3.2	3.3
		2.3	2.8	4.0	3.0
		3.4	3.3	3.0	3.0
		2.6	3.4	3.9	3.7
		2.6	2.0	3.4	2.6
		1.9	3.5	2.4	1.9
	Mean	2.8	3.0	3.4	3.0
Hydroxyproline solubilized, %		1.50	2.75	1.48	2.99
		1.59	2.61	1.32	1.98
		1.81	1.42	1.90	0.83
		1.46	2.04	1.83	2.00
		1.31	1.49	2.11	2.18
		2.20	1.26	1.58	1.25
		0.94	1.54	0.80	1.15
		1.17	1.29	0.76	1.31
	Mean	1.50	1.80	1.47	1.71

Table 18-Sensory scores for individual steaks[a]

Measurement		Oven temperature, °C			
		94°		149°	
		End point temp., °C		End point temp., °C	
		70°	80°	70°	80°
Tenderness		5.4	4.9	5.9	4.9
		6.0	5.3	4.6	4.4
		4.9	4.0	4.5	4.6
		5.6	4.6	5.5	5.3
		4.9	4.4	4.6	4.6
		5.8	4.2	5.0	3.8
		4.9	5.0	3.8	4.4
		4.8	4.2	4.9	4.6
	Mean	5.2	4.6	4.8	4.6
Softness		5.6	4.8	5.4	4.0
		6.1	5.6	3.4	3.4
		5.0	3.6	4.3	4.1
		5.4	3.1	3.9	4.4
		5.0	3.8	3.4	4.4
		4.9	3.8	4.4	2.9
		4.8	4.6	4.1	3.6
		4.5	3.1	4.3	4.4
	Mean	5.2	4.1	4.1	4.0

Table 18-(concluded)

Measurement	Oven temperature, °C			
	94°		149°	
	End point temp., °C		End point temp., °C	
	70°	80°	70°	80°
Mealiness	4.6	5.6	4.5	4.4
	4.5	5.4	3.9	5.0
	4.3	4.6	4.8	4.1
	4.5	3.6	3.9	4.9
	4.4	4.8	3.9	5.0
	4.6	4.3	5.0	3.5
	5.0	5.3	3.5	5.0
	4.3	4.1	3.5	4.1
Mean	4.5	4.7	4.1	4.5

[a]7-(extremely tender, extremely soft, extremely mealy);
1-(extremely tough, extremely hard, extremely chewy)

Table 19-Selected measurements on raw muscle

Replication number	Total moisture, %	Ether extract, %	pH
1	71.7	4.6	5.2
2	73.1	3.4	5.8
3	74.5	3.6	5.3
4	74.0	3.9	5.2
5	73.7	2.5	5.3
6	74.2	3.4	5.2
7	72.7	3.5	5.2
8	72.9	2.8	5.3
Mean	73.3	3.5	5.2

EFFECTS OF FOUR MOIST HEAT TREATMENTS ON COLLAGENOUS CONNECTIVE TISSUE IN BOVINE MUSCLE

by

Julia Leigh Rister
B. S., Texas Tech University, 1975

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Foods and Nutrition

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1976

Tenderness plays an important role in consumer acceptance of beef. A number of factors determine beef tenderness with the amount and distribution of collagenous connective tissue being one of the factors considered responsible for variation in tenderness. Since collagen is a major source of hydroxyproline, specific assays of this amino acid can be used to estimate the quantity of collagenous tissue in bovine muscle.

A randomized complete block design was followed to cook 32 top round steaks in oven film bags at 94° or 149°C to 70° or 80°C. Total cooking time ($P \leq 0.001$), drip cooking losses ($P \leq 0.001$) and softness scores ($P \leq 0.05$) were higher for steaks cooked at 94°C than for those cooked at 149°C. Measurements affected by end point temperature were total cooking time, total cooking losses and total moisture ($P \leq 0.001$) and drip cooking losses and sensory scores for tenderness and softness ($P \leq 0.01$). As total cooking time and total and drip cooking losses increased with an increased end point temperature, total moisture and sensory scores for tenderness and softness decreased. The rate at which heat penetrated the muscle was greater ($P \leq 0.001$) for steaks cooked at 149°C than for those cooked at 94°C. At oven temperature 94°C, end point temperature 80°C percentage hydroxyproline solubilized was correlated moderately with tenderness score ($r = 0.73^{*}$) and tenderness was highly correlated with shear value ($r = -0.83^{**}$) and with length of time the steak was held at an internal temperature of 55° to 60°C ($r = 0.86^{**}$). Values for total moisture were less ($P \leq 0.001$) and values for ether extract were greater ($P \leq 0.001$) in cooked than in raw muscle. There were no significant differences between oven temperatures or between end point temperatures for percentage hydroxyproline solubilized, shear value, ether extract or pH.