A COMPARISON OF THE CHEMICAL CHARACTERISTICS OF COLLAGEN AND ORGANOLEPTIC ATTRIBUTES OF GRAIN- VS. FORAGE-FINISHED CATTLE

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1978

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
July, 1983

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ACKNOWLEDGEMENTS

The author would like to extend sincere thanks to her major adviser, Dr. D. G. Wagner, for his valuable advice and assistance during the course of this study and preparation of this manuscript. Dr. J. E. Williams deserves special recognition for organizing this trial and for his helpful input in implementing research techniques and interpretation of the data. Thanks are also extended to Dr. R. L. Hintz for his helpful input with the statistical analysis of the data. Dr. T. H. Liao deserves special credit for the use of his amino acid analyzer and for his helpful advice on research procedures.

Technical support provided by Rebecca Ting and Mina Pan is sincerely appreciated. In addition, the frienship provided by Joe Ward, Kathy Emerson, Beth Omohundro, Chuck Hibberd, and Jim Williams will always be treasured.

Finally, this thesis is dedicated to my parents, Herbert and Anne Bulgerin, for their constant love, support, and encouragement during my graduate career.

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CHAPTER I

INTRODUCTION

Variations in sensory quality attributes of forage-finished vs. grain-finished beef are well documented (Bowling et al., 1977; Hufman and Griffey, 1975), but they are not well understood or explained. Some researchers report great differences in the quality of meat from cattle finished on grain vs. cattle finished on grass (Bowling et al., 1977; Kroft et al., 1975). However, other studies report no or very little differences in such attributes as tenderness, shear force, and connective tissue residue in the muscles from grain- vs. forage-finished cattle (Wu et al., 1981; Hunt and Hall, 1982).

Connective tissue is an integral component of muscle tissue which contributes to the tenderness of the muscle. It has been reported that as an animal ages the collagen of intramuscular connective tissue increases in stability and changes from an easily solubilized protein into an insoluble fiberous connective tissue network (Bailey, 1969; Shimokomaki et al., 1972; Dutson, 1974). The conversion of soluble collagen to insoluble collagen is caused by the formation of stabilizing intermolecular crosslinks along the collagen

fibers at certain hydroxylsine, lysine, and histidine residues.

The purpose of this study was to evaluate and compare forage-finished and grain-finished beef for organoleptically detectable differences in tenderness and connective tissue residue in longissimus dorsi and semitendinosus muscles, and to analyze the chemical properties of collagen in the muscle tissue of the animals from the two feeding treatments to determine whether there were any differences in the hydroxylysine, lysine, and histidine content in total and insoluble collagen.

CHAPTER II

LITERATURE REVIEW

Beef is the most popular red meat consumed in the United States today. Except for hamburger from non-fed beef, the majority of beef available to the consumer has been finished on high-grain rations. As the population of the world increases there will probably be an increasing demand to use world grain production to feed humans. It is likely that at sometime in the future it will not be possible to finish cattle on grain as extensively or for as long a feeding period as is now the case. One alternative is a more extensive use of forages in the growing and finishing rations of cattle.

Organoleptic Evaluations

Several researchers have evaluated the differences in sensory characteristics between forage- and grain-finished beef. Bowling et al. (1977) found that longissimus dorsi muscles from conventionally-chilled, forage-finished carcasses were ($P<.05$) less tender, had ($P<.05$) greater quantities of organoleptically-detectable connective tissue and had ($P<.05$) higher shear force values than muscles from conventionally-chilled, grain-finished carcasses.

Kroft et al. (1975) compared palatability characteristics of meat from long-fed, short-fed, and grass-fed cattle. They found that only 40% of the steaks from grass-fed cattle had acceptable tenderness ratings, whereas 90% of the steaks from the short-fed and 100% of the steaks from the long-fed cattle were acceptable.

Hufman and Griffey (1975) evaluated forage-fed and grain-fed beef. Contrary to the two previous reports, they found no significant differences in tenderness of the steaks between the two feeding treatments. All the steaks evaluated were rated well above the acceptable level of tenderness.

Wheeling, Berry, and Carpenter (1975) compared forage- and grain-finished beef and found that the shear force values were lower and the tenderness ratings were significantly higher for steaks from forage-fed cattle than steaks from high-quality grain-fed or low-quality grain-fed cattle. The authors indicated that the increased tenderness of the steaks from forage-finished cattle may have been due to the forage-finished cattle being more youthful than the grain-finished cattle.

At Kansas State University, Wu et al. (1981) ran a trial to determine effects of different nutritional regimens on beef palatability and collagen characteristics. The researchers finished one group of cattle on grass, a second group on a high-energy diet for 120 days, and a third group which was left on grass for an extended period and then finished on a high-energy diet. The results of the taste panel

study showed that the longissimus dorsi steaks from the three groups did not differ in total collagen content or in rating for tenderness, juiciness, and flavor intensity. The intramuscular collagen content of the longissimus dorsi muscle did not vary among the three groups; however, longissimus dorsi samples from group 2 had a higher ($P<.05$) percentage of salt-soluble collagen than the grass-finished group. Animals from group 3 had higher ($P<.05$) percentages of acid-soluble and acid-plus-salt-soluble collagen than did samples from groups 1 and 2. There were no significant differences in collagen solubility nor intramuscular collagen content of the biceps femoris muscle among the three groups of cattle.

In another study at Kansas State University, Hall and Hunt (1982) evaluated the effect of nutritional regimen on collagen solubility. In young cattle which are growing rapidly collagen lacks extensive crosslinking because the collagen is in a stage of rapid biosynthesis coinciding with muscle growth. As the growth of the animal slows, the collagen biosynthesis slows and the collagen begins to mature by forming stabilizing crosslinks. Increased crosslinking causes the collagen to change from a relatively soluble form into a more insoluble resilient fiber. This in turn could have an effect on the tenderness of the muscle because of the increased amounts of insoluble collagen added to the connective tissue residue. In general, only small differences were noted in total collagen content of longissimus dorsi muscles from forage- vs. grain-finished beef. Diet did not have a

consistent effect on salt-soluble, acid-soluble, and salt-plus-acid-soluble collagen. This group concluded that collagen is more subject to crosslinking in the muscle of cattle fed high-energy diets and subsequently switched to diets that restrict energy intake. They also hypothesize that cattle fed roughage diets may grow slower and reach a mature weight at an older age; consequently, their collagen solubilities may not decrease until they are chronologically older than concentrate-fed cattle.

The variability in the results obtained indicates that many different factors associated with tenderness may be involved.

Beef Tenderness

There are several factors which contribute to the tenderness of beef. Among these are the age of the animal at slaughter, the contractile state of the muscle at the time of rigor mortis, and the chemical characteristics of the collagen of intramuscular connective tissue. If the collagens in meat possess thermally-stable crosslinks the collagen is insoluble and the fibers retain a significant proportion of their strength following denaturation. The residual strength of the collagen fiber is sufficient to maintain the adhesion of the muscle fibers. On the other hand, if the bonds are thermally labile, the denatured collagen possesses little tensile strength and partially dissolves in the muscle fluid, thus further reducing the adhesion of the myofibers, which results

in the meat being tender when cooked (Bailey and Sims, 1977; Cover et al., 1962; Locker, 1963; McCrae et al., 1974; Dutson et al., 1976; Marsh et al., 1966; Marsh, 1977). Dutson et al. (1976) showed that in muscles which contain reduced quantities of collagen, cold shortening would have the major toughening effects. Results indicated that connective tissue content plays a role in the overall tenderness of a muscle at all sarcomere lengths and it also contributes to the amount of toughening a muscle undergoes when it shortens.

The method of cooking (dry versus moist heat), the duration of cooking time, and temperature can all have an effect on the tenderness of meat (Cover et al., 1962; Leander et al., 1978).

The contractile proteins of muscle also contribute significantly to the tenderness of the meat. Visible red muscles are stimulated to shorten if exposed to cold temperatures (10^{o}C) while still in a pre-rigor state (Marsh, 1977). This cold shortening effect is especially evident in carcasses containing less than .3 inch of fat cover. Therefore, the lean carcasses produced from forage-finished cattle may be more susceptible to cold shortening than those from grain-finished cattle. It is possible to minimize cold shortening effects by hanging the carcass in such a way that the muscle is stretched and prevented from shortening. Another method available to prevent cold shortening is electrical stimulation of the carcass which rushed the musculature into rigor

at a very high rate. When carcasses have been managed correctly the major factor contributing to the tenderness variation in muscles is probably the collagen of the intramuscular connective tissue.

Intramuscular Collagen

Connective tissue is classified as the epimysium, the perimysium, and the endomysium. The epimysium forms the outer sheath of the muscle, the perimysium surrounds the muscle bundles, and the endomysium surrounds each individual muscle fiber.

Collagen is a protein which comprises approximately one-fourth of all the body protein. The role of collagen in the body is primarily a structural one, participating in support and interconnection of muscle tissues. It is able to fulfill this function due to its unique molecular configuration and arrangement of amino acids. Through specific aggregation and crosslinking, these molecules form fibrils of unusual strength and stability (Traub and Piez, 1971).

The tropocollagen molecule is the structural unit of the collagen fiber (Veis et al., 1965; Dutson, 1976). It consists of three polypeptide chains, known as α chains, which are intertwined around each other, forming a rodlike helical structure about 3000 A long and 15 A wide (Bailey, 1969). There are three different types of α chains that can be present in the tropocollagen molecule called α1, α2, and α3. The α1 and α2 strands are most predominant in intramuscular

collagen, and a system of intramolecular crosslinkages exists so that various strand dimers, called β units, and trimers, called γ units, may exist (Veis et al., 1965). Collagen molecules are synthesized in fiber-forming cells, called fibroblasts, as procollagen. Following synthesis of the polypeptide chains the procollagen goes through many post-translational modifications which include hydroxylation of selected lysine and proline residues by the enzymes lysine hydroxylase and proline hydroxylase, respectively (Miller et al., 1970), glycosylation of certain hydroxylysine residues, and oxidative deamination of certain lysine and hydroxylysine residues to yield the aldehydes allysine and hydroxyallysine which are precursors of intermolecular collagen crosslinks. Just prior to extrusion from the fibroblast the procollagen undergoes proteolytic cleavage to tropocollagen. The amino acid sequence of tropocollagen is unique in that every third residue is glycine, and there is a repeating tripeptide gly-x-y in which x and y are frequently hydroxyproline and proline, respectively (Traub and Piez, 1971; Bailey and Robins, 1976; Dustson, 1976). The molecule is intertwined so that glycine is always located on the inside of the molecule where it participates in intramolecular H-bonding. The pyrrolidine rings of proline and hydroxyproline are on the outside of the molecule and are responsible for the formation of the polyproline II helix that is characteristic of the tropocollagen molecule (Bailey and Robins, 1976; Dutson, 1976). There is a telopeptide region at both the N- and C- terminal ends of

the α chains which do not contain glycine as every third amino acid, and hence, these regions do not participate in the helical arrangement of the rest of the molecule. The tropocollagen molecules form fibrils by specific aggregation and crosslinking (Traub and Piez, 1971).

Bailey et al. (1977) determined that intramuscular collagens are not identical. Their research revealed that epimysium contained mainly Type I collagen, perimysium contained mainly Type III collagen, and endomysium contained both Types I and III collagen.

Type I collagen contained two identical chains (α1) and a chemically different third chain (α2). Type III collagen is composed of three chemically identical chains (α3). Both Type I and Type III collagen contains 6-8 hydroxylysine residues per 1000 amino acids in the chain (Bailey et al., 1976). The discovery of these genetically distinct collagens suggests that different cell types may be involved in the biosynthesis (Bailey and Robins, 1976).

Collagen Crosslinks

Two types of crosslinking are known to occur in collagen which contribute to fibril formation. Intramolecular crosslinks occur within the tropocollagen molecule (Bornstein and Piez, 1966) and intermolecular crosslinks occur between the molecules in the intact fiber (Shimokamaki et al., 1972).

The intramolecular crosslink found in soluble collagen occurs at specific lysyl residues at both the NH_2 and

C-terminal and non-helical regions of the individual α chains. These crosslinks join together either two adjacent α1 polypeptide chains or an α1 and α2 polypeptide chain when lysyl residues from each chain become oxidatively deaminated to form α-aminoadipic δ-semialdehyde residues which then condense to form the crosslink (Bornstein and Piez, 1966); Kang et al., 1969; Rojkind et al., 1969). The formation of this aldol condensation product is spontaneous (Piez, 1970).

The intermolecular crosslinks are more important than the intramolecular crosslinks in the stabilization of the collagen fibers (Shimokomaki et al., 1972). Extensive analyses have demonstrated that they are aldimine-type bonds (Bailey, 1969). To date, three major aldimine-type crosslinks have been isolated from borohydride-reduced collagen. One crosslink, lysinonorleucine (Tanzer, 1970; Kang et al., 1970), arises from a residue of allysine and a residue of lysine, and would have the formula

$$P\text{-}CH_2\text{-}CH_2\text{-}\underset{OH}{CH}\text{-}CH_2\text{-}N = CH\text{-}CH_2\text{-}CH_2\text{-}CH_2P'$$

in its unreduced form. The third crosslink, hydroxylysinohydroxynorleucine, has been shown to be derived by condensation of a hydroxylysine aldehyde with another hydroxylysine aldehyde (Davis and Bailey, 1971) and would have the formula

$$P\text{-}CH_2\text{-}CH_2\text{-}\underset{OH}{CH}\text{-}CH_2\text{-}\overset{H}{N}\text{-}CH_2\text{-}\underset{OH}{CH}\text{-}CH_2\text{-}CH_2\text{-}P'.$$

Shimokomaki et al. (1972) observed an increase in the proportion of the reducible crosslinks from the fetal stage to a maximum at 12-18 months. This period coincides with a rapid rate of growth and the formation of labile intermediate crosslinks. It is also the period when collagen is most soluble. This is due to the presence of non-covalently-bound neutral salt-soluble collagen and the susceptibility of the labile reducible crosslinks to cleavage by dilute organic acids. In all the muscles and tendons they studied there was a gradual decrease in the proportion of all three reducible crosslinks at 12 to 18 months, until they were virtually absent at maturity. This confirms the proposal (Bailey, 1969) that all three are intermediates in the crosslinking process and are gradually stabilized with increasing age. Shimokomaki et al. (1972) proposed that the decrease of collagen solubility with increasing age must be due to the decrease of overall rate of synthesis of new collagen. This would give time for the labile bonds to stabilize themselves by conversion to a nonreducible form, creating thermally-stable crosslinks which render the collagen insoluble. As the proportion of stable crosslinks increases with age, the heat-denatured collagen fibers in the cooked meat become stronger and thus make a greater contribution to the tenderness of the meat. The studies by Shimokomaki et al. (1972) suggest that the tenderest beef should be obtained at about 12-14 months.

CHAPTER III

A COMPARISON OF THE CHEMICAL CHARACTERISTICS OF COLLAGEN AND ORGANOLEPTIC ATTRIBUTES OF GRAIN- VS. FORAGE-FINISHED CATTLE

Summary

During two consecutive years, 20 yearling Brangus X Hereford-Angus steers (348 kg) per year were randomly assigned to: 1) a conventional high concentrate finishing ration composed of corn grain for approximately 160 days, or 2) a forage finishing system utilizing sorghum-sudan and winter wheat pasture for 190 days prior to slaughter. When data were adjusted to a constant hot carcass weight (HCW), dressing percentage, fat cover, marbling score and quality grade were higher ($P<.05$ or $P<.01$) for steers finished on grain than those finished on forage.

Longissimus dorsi (LD) and semitendinosus (ST) steaks from the grain-finished carcasses had higher ($P<.05$) tenderness scores and lower connective tissue residue ratings by taste panelists than those from the forage-finished group. Collagen content was higher in the semitendinosus muscle

(2.89 and 2.53 mg collagen/g of wet tissue) and lower in the longissimus dorsi muscle (1.41 and 1.64 mg collagen/g of wet tissue) of grain-finished than forage-finished cattle. There were no consistent differences between feeding regimens in hydroxylysine, lysine, and histidine content of total and insoluble collagen.

(Key Words: Collagen, Cattle, Production Systems, Meat Palatability, Amino Acids.)

Introduction

The use of more forages and less grain in beef production systems may be needed in the future. Reasons may include a need for energy conservation, potentially greater world demand for grain for both human and livestock consumption, increasing consumer desire for leaner meat and a better understanding of the relationships between dietary factors and human health. Variations in sensory quality attributes of forage-finished versus grain-finished beef are well documented (Bowling et al., 1977; Hufman and Griffey, 1975), but the reasons for these variations are not fully understood.

Tenderness is one of the most important quality attributes of beef palatability. Beef tenderness can be divided into three components: actomyosin effects, intramuscular fat and moisture effects, and background effects which are due to the quantity and/or chemical state of the intramuscular connective tissue of which collagen is a major component.

It has been shown by many researchers (Bailey, 1969; Shimokamaki, 1972; Dutson, 1974) that as an animal ages, and especially after physiological maturity is reached, the collagen of connective tissue converts from an easily solubilized protein to a resilient insoluble form due to the formation of intermolecular crosslinks. Therefore, as the beef animal increases in age, the tenderness of the meat tends to decrease with the increasing amounts of insoluble collagen in the muscle tissue. The main amino acids participating in formation of these insoluble collagen crosslinks are lysine, hydroxylysine and, to some extent, histidine. Of these amino acids, hydroxylysine is found almost exclusively in collagen protein and not in the other body proteins. This should make hydroxylysine an ideal amino acid to use in the determination of the total amount of collagen in intramuscular connective tissue.

The purpose of this study was to evaluate and compare forage-finished and grain-finished beef for organoleptically detectable differences in tenderness and connective tissue residue, and to analyze the chemical properties of collagen in the muscle tissue of animals from the two feeding treatments to determine whether there were any differences in the hydroxylysine, lysine, and histidine content in total and insoluble collagen.

Materials and Methods

During two consecutive years, 40 fall-born Brangus X

Hereford-Angus steers (20 steers per year, weaned at approximately 10 months of age) were assigned randomly to one of two feeding regimens. Twenty steers were placed in confinement and fed a high concentrate corn grain (79% corn) diet (Table I) for approximately 160 days. The other 20 steers were grazed, after weaning (July), on a high-quality sorghum-sudan mix followed by a winter wheat pasture which was grazed for the majority of the finishing period of approximately 190 days. Prior to weaning, the cows and calves were managed on a complementary forage program, utilizing creep grazing of wheat pasture for the calves during the winter, followed by a high-quality sorghum-sudan and bermuda-grass pasture. All the steers were of a similar weight (approximately 348 kg) at the start of the trial. Slaughter end points were based on the grain-finished cattle grading low choice (USDA, 1976) and the forage-finished cattle grading good, which coincided with the end of the forage production system. Different slaughter end points were chosen in an attempt to minimize differences in both carcass quality and animal age at slaughter. All initial and final weights were taken after removal from feed and water overnight.

After the cattle were slaughtered, hot carcass weight (HCW) and dressing percentage were determined. The carcasses were chilled for 48 hours or longer at 0 $\pm$ 1^{o} C, then evaluated for marbling, maturity, quality grade, fat thickness, ribeye area, percentage kidney, heart and pelvic (KHP) fat and yield grade (USDA, 1976). The longissimus dorsi muscle

TABLE I

HIGH CONCENTRATE RATION COMPOSITION[ab]

Item	International Feed Number	Percent
Corn	4-02-931	79.0
Cottonseed hulls	1-01-599	5.0
Alfalfa meal	1-00-023	5.0
Molasses, cane	4-04-696	5.0
Soybean meal	5-04-600	4.0
Urea	5-05-070	.7
Salt (plain)	6-04-152	.5
Dicalcium phosphate	6-01-080	.4
Calcium carbonate		.4

[a]Vitamin A Supplement (30,000 IU/g) added at .015% of the ration.

[b]Dry matter basis.

from the short loin at the 13th rib and the semitendinosus muscle from the round were excised from the right side of the carcasses, wrapped in polylaminated wrapping paper and stored at -40° C for later evaluation by taste panelists. The steaks for collagen analyses were well trimmed to remove external fat and epimysial connective tissue. The muscle tissue was then diced, ground through an eighth-inch (0.32 cm) plate and frozen.

Crude intramuscular collagen was extracted from the samples using the procedure of Wu and Dutson (1977), which is a modification of the procedure of Field (1970). A 25-gram sample of ground muscle was extracted twice with 125 ml cold distilled water and three times with 0.1 M potassium phosphate buffer pH 7.4 containing 1.1 M potassium iodide by blending 15 seconds for each extraction in an Omni mixer. The homogenates were filtered through cheese cloth to retain the connective tissue. The crude preparation of intramuscular connective tissue was then washed in a large volume (approximately 2000 ml) of 1 M potassium chloride to remove traces of myofibrillar proteins and then in 0.9% solution of sodium chloride to remove potassium ions from the sample. The samples were washed with distilled water to remove the sodium chloride and centrifuged at 4000 times G for 5 minutes and the supernatant decanted. The final isolated connective tissue was then lypholyzed to remove all moisture, the weight recorded and the samples stored in the freezer (0° C) until analyzed.

Total collagen content of the isolated connective tissue was determined on 10 randomly selected longissimus dorsi and semitendinosus muscle samples from each treatment, using an amino acid analyzer. The decision to analyze only one-half of the connective-tissue samples on the amino acid analyzer was based on the cost and time involved in sample analysis. Approximately 500 microgram portions of lypholyzed crude intramuscular connective tissue were placed in hydrolysis tubes. To each sample was added 0.2 ml of 6N HCl. The tubes were evacuated with a vacuum and placed in a 100^{o} C oven for 24 hours. The samples were then dried in a dessicator under vacuum and redissolved in 0.5 ml sodium citrate buffer (Appendix, Table VIII). A 0.2 ml aliquot of the dissolved connective tissue was run through the short column (15 cm) of the amino acid analyser (Beckman Model 120G) using sodium citrate buffer as the running buffer and Ninhydrin reagent (Appendix, Table VIII) for color development. Absorbence was read at 440 nm and 570 nm. The amino acids quantified were hydroxylysine, lysine, and histidine. Standard solutions of Type I and Type III bovine collagens were also prepared according to the above procedure. The total collagen in each connective tissue sample was determined using hydroxylysine as the standard amino acid.

Soluble collagen was determined using the spectrophotometric method described by Goll (1964). Duplicate 2 mg samples of lypholyzed intramuscular connective tissue were weighed into 15 ml centrifuge tubes. To each tube were added

2 ml of 0.1 M sodium phosphate buffer, pH 7.0. The tubes were incubated in a water bath at 69° C for one hour. The samples were cooled then centrifuged at 6000 rpm for 15 minutes. Just prior to analysis, the copper reagent was prepared by mixing 1 ml of 0.05 M copper sulphate with 1.5 ml of 2.5% sodium tartrate. Two ml of this mixture were then diluted to 25 ml with an 8% sodium carbonate solution in 0.2 N sodium hydroxide. One ml of copper reagent was added to 1 ml of sample supernatant, mixed immediately and allowed to stand at room temperature for at least 10 minutes. Dilute Folin-Ciocalteu reagent (0.2 ml of 1 N) was added and immediately mixed. The samples were allowed to stand at room temperature for 45 minutes and the absorbencies were read at 750 nm. A gelatin standard (Appendix, Table IX) was used for the standard curve. Percent insoluble collagen was determined by difference. The insoluble collagen pellet remaining after the soluble protein procedure was washed with distilled water, lypholyzed, and analyzed for amino acid composition following the same procedure previously described for the total collagen analysis.

Organoleptic evaluation of longissimus dorsi and semitendinosus steaks were conducted by L. E. Ebro, Oklahoma State University, Department of Home Economics. The palatability score sheet used in the sensory evaluation was adapted from a similar score sheet developed by the Meat Science Research Laboratory, USDA (Appendix, Table X).

Following an intial training period, nine sensory panelists sampled cubes of prepared steaks cut from 20 different carcasses, with 10 of the carcasses having been randomly selected from among those of each group of 20 steers which had been raised under the 2 feeding regimens.

All steaks were prepared (void of seasonings) on a Farberware "Open Hearth" broiler (No. 450n). To insure that all the steaks reached the same degree of doneness, a thermocouple was inserted in the center of each steak and the internal temperature was monitored on a Honeywell Multipoint Temperature Recorder Electronic 111/112. The steaks were cooked to an internal temperature of 69^{o} C. Nine trained taste panelists evaluated six sensory characteristics pertaining to the palatability of the steaks: initial juiciness, sustained juiciness (8 chews), tenderness (first impression), amount of connective tissue (15 chews), beef flavor intensity, and off-flavor intensity. Three of these quality attributes, tenderness, amount of connective tissue, and off-flavor intensity, are reported in this paper. Data were analyzed by analysis of variance for significance of differences between treatments (Steel and Torrie, 1968).

Results and Discussion

Carcass Characteristics

Steers finished on grain had higher final weights, total weight gain, and average daily gains ($p < .01$) than steers

finished on forage (Table II). The higher daily gains of steers finished on grain in comparison to wheat pasture resulted in cattle finished on grain being slaughtered in 51 fewer days. The steers on wheat pasture had to contend with light rainfall during part of the trial, resulting in somewhat lower gains than anticipated. Carcasses from the steers finished on grain had heavier ($p<.01$) hot carcass weights, higher ($p<.01$) dressing percentages, marbling scores and quality grades, greater ($p<.05$) twelfth rib fat thickness, and maturity scores than the steers finished on wheat pasture. The longissimus muscle areas were larger and the yield grades lower in the carcasses of the grain-than the forage-finished steers (Table III). The steers finished on grain graded 65% choice and 35% good, while those on forage graded 5% choice, 50% good, and 45% standard. The uniformity of the grain-finished as compared to the forage-finished steers indicates that possibly the inconsistency and unpredictability of the environment of forage-finished cattle may be one of the major problems associated with forage-finishing programs.

Organoleptic Evaluation

Taste panel evaluations of longissimus dorsi and semitendinosus steaks revealed no difference ($p<.05$) in tenderness between grain-finished and forage-finished semitendinosus samples; however longissimus dorsi steaks from the grain-finished steers were more tender ($p<.05$) than those from the forage-finished steers (Table IV). These findings agree with

TABLE II

PERFORMANCE OF FORAGE- VS. GRAIN-FINISHED BRANGUS X HEREFORD-ANGUS STEERS

Item	Forage	Grain	SE
Number of animals	20	20	
Initial wt, kg	349.6	332.5	
Final wt, kg	441.8[a]	482.1[b]	6.4
Total gain, kg	92.2[a]	149.6[b]	5.6
Days on experiment	196	145	
Daily gain, kg	.47[a]	1.12[b]	.04

[ab]Means in the same row with different superscripts differ at $P<.01$.

TABLE III

CARCASS CHARACTERISTICS OF FORAGE- VS. GRAIN-FINISHED BRANGUS X HEREFORD-ANGUS STEERS

Item[a]	Forage	Grain	SE
Hot carcass wt, kg	239.8[b]	299.5[c]	.58
Dressing percentage	54.0[b]	61.0[c]	.58
Maturity[f]	13.7[d]	14.3[e]	.16
Marbling score[g]	9.4[b]	15.0[c]	1.14
USDA quality grade [h]	8.6[b]	12.4[c]	.51
Longissimus muscle area, cm^2, [i]	66.5	70.7	3.34
Fat, cm[i]	0.7[d]	1.5[e]	.16
USDA yield grade	2.67	3.37	.26

[a]All parameters except hot carcass wt (HCW) were adjusted to the mean of the HCW (269.7 kg) for both treatments.

[bc]Means in the same row with different superscripts differ at P<.01.

[de]Means in the same row with different superscripts differ at P<.05.

[f]15 = A-; 14 = A; 13 = A+.

[g]g = traces; 12 = slight; 15 = small.

[h]6 = low standard; 9 = low good; 12 = low choice.

[i]Twelfth rib.

TABLE IV

ORGANOLEPTIC EVALUATION OF LONGISSIMUS DORSI (LD) AND SEMITENDINOSUS (ST) STEAKS FROM FORAGE VS. GRAIN-FINISHED BEEF[d]

Sensory Attribute	Muscle	Treatment		SE[d]
		Forage	Grain	
Tenderness[e]	LD	4.60^{ab}	5.45^{c}	0.24
	ST	4.20^{a}	5.09^{bc}	0.24
Connective tissue residue[f]	LD	4.34^{ab}	5.18^{c}	0.23
	ST	3.91^{a}	4.64^{bc}	0.23
Off-flavor intensity[g]	LD	6.89^{ab}	7.19^{c}	0.10
	ST	6.87^{a}	7.16^{bc}	0.10

[abc]Means in a row or column with different superscripts differ statistically ($P<.05$).

[d]Values are the means of 10 observations $\pm$ the standard error (SE) of the mean)

[e]8 = Most Tender; 1 = Least Tender.

[f]8 = Least Abundant; 1 = Most Abundant.

[g]8= No Off-Flavor; 1 = Extremely Intense Off-flavor.

those of Bowling, et al. (1977) and Kroft, et al. (1975), who also report longissimus dorsi steaks from grain-finished cattle more tender ($p<.05$) than those from forage-finished cattle. In contrast, Wheeling, et al. (1975) rated steaks from forage-finished cattle more tender ($p<.05$) than similar steaks from high-quality grain-finished and low-quality grain-finished cattle. The authors indicated that these reults may have been influenced by the cattle finished on forage being more youthful than the cattle finished on grain. In the studies conducted by Wu, et al. (1981) and Huffman and Griffey (1975) no differences ($p<.05$) were found between the tenderness ratings of cattle finished on forage versus cattle finished on higher energy diets. The lack of similarity between studies indicates that more research is necessary to determine why there is so much variation in tenderness of forage-finished beef. Factors such as the age and maturity of the animal at slaughter, the chemical maturity of the intramuscular collagen at slaughter, and the quality, abundance and type of forage used in the finishing system may all have an effect on muscle tenderness.

Evaluation of connective tissue residue by taste panelists revealed longissimus dorsi steaks from grain-finished cattle had less ($p<.05$) connective tissue residue than the longissimus dorsi steaks from the forage-finished cattle (Table IV). Again no significant differences were found between feeding regimens for the semitendinosus muscles. These results agree with those of Bowling, et al. (1977),

who also found greater ($p<.05$) amounts of organoleptically-detectible connective tissue residue in longissimus dorsi muscles of forage- vs. grain-finished cattle.

The off-flavor intensity was greater ($p<.05$) in both the longissimus dorsi and semitendinosus muscles from forage-finished than from grain-finished cattle (Table IV). These results differ from those of Wu, et al. (1981), who found no difference in the flavor of grain- and forage-finished beef.

Intramuscular Collagen

There were no within-treatment differences ($p<.05$) in the total collagen, soluble collagen, and insoluble collagen content of longissimus dorsi and semitendinosus muscles from grain- and forage-finished cattle (Table V). There were greater amounts ($p<.05$) of total and insoluble collagen in the semitendinosus muscles than in the longissimus dorsi muscles of forage-finished than grain-finished cattle (Table V). These findings agree with Wu, et al. (1981), who found no difference ($p<.05$) in the total collagen content of longissimus dorsi and biceps femoris samples from forage- and grain-finished cattle. However, Wu, et al. (1981) reported a higher ($p<.05$) percentage of soluble collagen in the longissimus dorsi muscle from the grain- than forage-finished cattle. This difference was not present in the bicep femoris muscle. Hall and Hunt (1982) also reported no differences ($p<.05$) in total and soluble collagen present in longissimus dorsi muscles from forage- and grain-finished cattle. It was

TABLE V

EFFECTS OF FORAGE- VS. GRAIN-FINISHED BEEF ON COLLAGEN COMPOSITION OF LONGISSIMUS DORSI (LD) AND SEMITENDINOSUS (ST) MUSCLES[c]

Item	Muscle	Treatment		SE
		Forage	Grain	
Collagen Content (mg/g Fresh Tissue)	LD	1.64[a]	1.41[a]	±0.35
	ST	2.53[b]	2.89[b]	±0.35
Soluble Collagen (mg/g Fresh Tissue)	LD	0.40	0.33	±0.04
	ST	0.33	0.44	±0.04
Insoluble Collagen (mg/g Fresh Tissue)	LD	1.24[a]	1.08[a]	±0.04
	ST	2.20[b]	2.44[b]	±0.04

[ab]Means in a row or column with different superscripts differ statistically ($P<.05$).

[c]Values are the means from 20 observations ± the standard error of the mean.

noted in this study that the amounts of total collagen extracted from the individual muscle samples from both grain- and forage-finished cattle were highly variable. Possibly the collagen extraction procedure used was not as precise a method as could be developed; losses of small amounts of intramuscular connective tissue on the cheesecloth could not be avoided. However, more research is necessary to determine the mechanisms of intramuscular collagen biosynthesis to discover why some animals produce more collagen than others. Also, values could be confounded by irregular grouping of collagen fibers throughout the muscle being analyzed.

Amino Acid Analysis

In this study, the amino acids hydroxylysine, lysine, and histidine were quantitated in the total intramuscular collagen sample extracted from the muscle tissue and in the insoluble collagen residue to determine if there were any differences in the amino acid composition of the total and insoluble collagen between the two feeding regimens.

There were no differences ($p<.05$) in the percentages of hydroxylysine, lysine, and histidine in the total collagen extracted from the longissimus dorsi and semitendinosus muscle samples (Table VI). This data agrees with Bailey, et al. (1977), who reported the presence of uniform proportions of type I and type III collagen in muscle tissue. There was a greater ($p<.05$) percentage of hydroxylysine present in the insoluble collagen of semitendinosus muscle than

TABLE VI

EFFECTS OF FORAGE- VS. GRAIN-FINISHED BEEF ON THE PERCENTAGE OF HYDROXYLYSINE, LYSINE, AND HISTIDINE IN THE TOTAL AND INSOLUBLE COLLAGEN FROM LONGISSIMUS DORSI (LD) AND SEMITENDINOSUS (ST) MUSCLES[c]

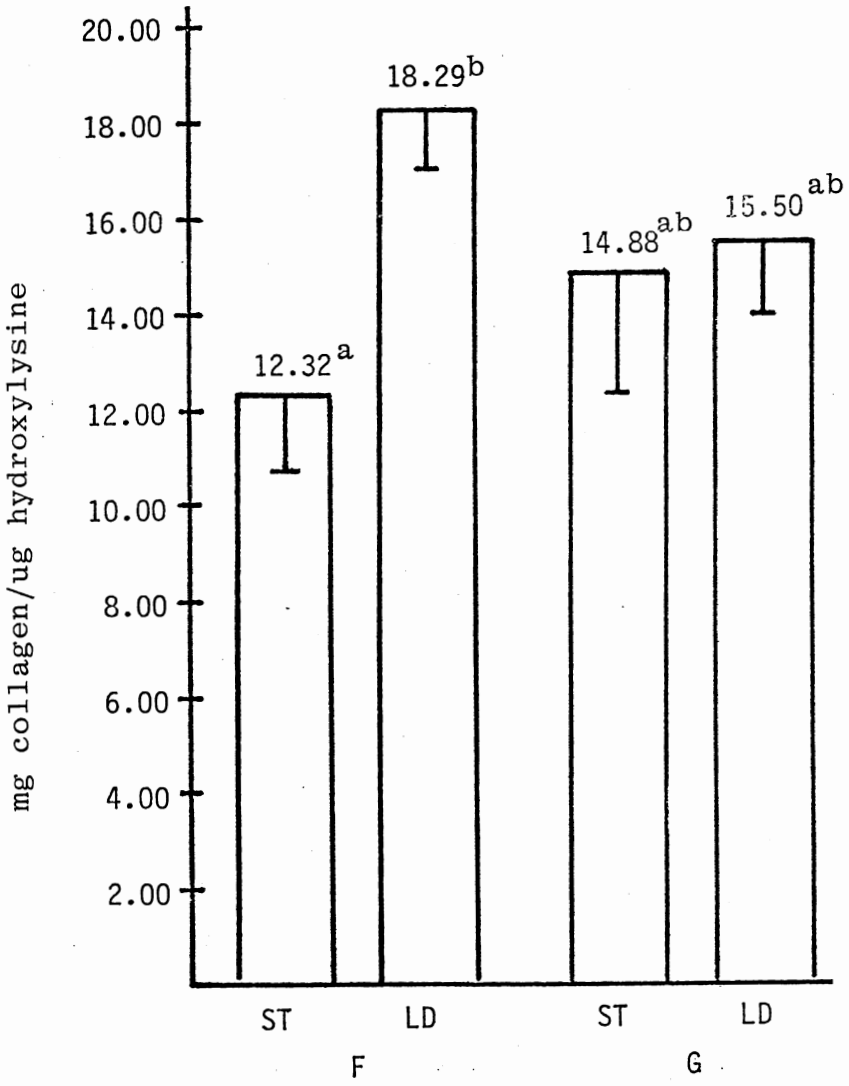
Treatment	Muscle	% Hydroxylysine		% Lysine		% Histidine	
		Total Collagen	Insoluble Collagen	Total Collagen	Insoluble Collagen	Total Collagen	Insoluble Collagen
Forage	LD	1.17	0.92[b]	3.76	3.44	1.14	1.10
Finished	ST	1.17	1.51[a]	3.69	3.87	1.18	1.35
Grain	LD	1.17	1.15[b]	3.79	3.72	1.18	1.18
Finished	ST	1.28	1.32[a]	4.41	3.98	1.44	1.33
Standard Error		±0.01	±0.12	±0.14	±0.30	±0.04	±0.11

[ab]Means in a column with different superscripts differ statistically ($P<.05$).

[c]Values are the means of 10 observations ± the standard error of the mean.

longissimus dorsi muscle in both the forage-finished and grain-finished cattle.

Two types of crosslinking occur in collagen: intramolecular crosslinks which occur within the tropocollagen molecule (Bornstein and Piez, 1966) and intermolecular crosslinks which occur between collagen molecules (Shimokomaki, et al., 1972). Hydroxylysine, lysine, and histidine are the primary amino acids which participate in the intermolecular crosslinking and stabilization of the collagen fiber. The ratio of these three amino acids in the insoluble collagen as compared to the total collagen may be some indication of the amount of crosslinking which has occurred in the collagen and, therefore, may relate to the tenderness of the muscle. To visualize this effect, the ratio of the amount of insoluble collagen containing one microgram of the amino acids hydroxylysine, lysine, and histidine were calculated. The most marked effect was with hydroxylysine, which was most concentrated in the semitendinosus muscle and least concentrated ($p<.05$) in the longissimus dorsi muscle from forage-finished beef (Figure 1). If concentration of crosslink precursor amino acids in insoluble collagen does play a role in muscle tenderness, then from this data one would expect the longissimus dorsi steaks from the forage-finished cattle to be the most tender, followed by longissimus dorsi steaks from the grain-finished beef, then the semitendinosus steaks from the grain-finished and forage-finished beef to be the least tender, respectively. However, comparing the data with



[ab]Means with different superscripts differ statistically ($P<.05$).

Figure 1. Ratio of insoluble collagen to hydroxylysine in longissimus dorsi (LD) and semitendinosus (ST) muscles from forage- (F) and grain-finished (G) beef (10 observations/mean).

the taste panel evaluation (Table IV), it is apparent that this this is not the order of tenderness determined by the taste panel. The semitendinosus steaks from the forage-finished beef were the least tender, as was expected, but the taste panel evaluated the longissimus steaks from the forage-finished group, the sample with the lowest concentration of hydroxylysine, to be less tender than both the longissimus and semitendinosus steaks from the grain-finished group.

The ratio of the amount of insoluble collagen containing one microgram of lysine and histidine were very similar in longissimus dorsi and semitendinosus muscles from both feeding regimens (Figures 2 and 3). The results of this data indicate there is limited, if any, relationship of muscle tenderness to concentration of amino acids participating in intermolecular collagen crosslinks in the muscles evaluated. It is possible that the steers used in this study had not reached the maturity stage when the majority of reducible crosslinks had stabilized into the non-reducible form (Shimokomaki, et al., 1972; Bailey, 1969), causing the data to be inconclusive.

It is difficult to determine the real effects of collagen's chemical characteristics on muscle tenderness because there are too many factors that contribute to tenderness which are difficult to eliminate, such as the stress on the animal at the time of slaughter, the contractile state of the muscle at the time of rigor mortis, and the effects of cold shortening on the muscle fibers. It is also important to realize that collagen is a necessary component of meat; without it

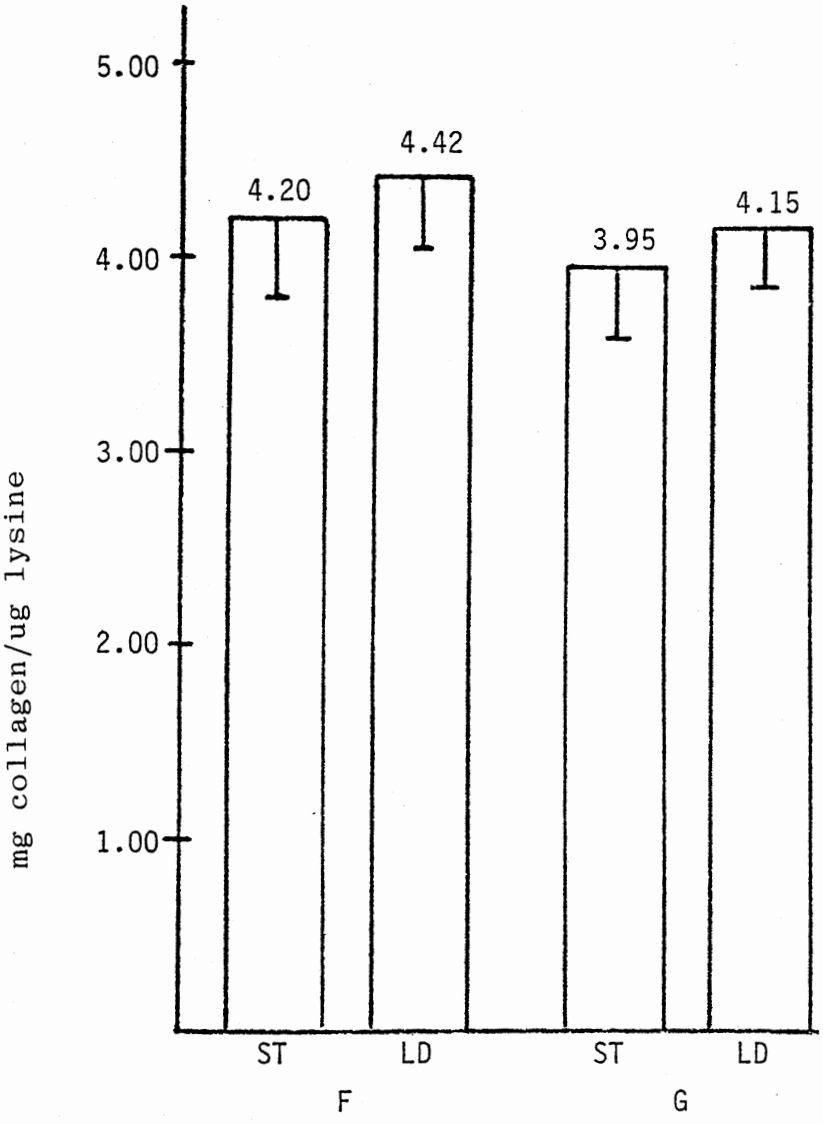


Figure 2. Ratio of insoluble collagen to lysine in longissimus dorsi (LD) and semitendinosus (ST) muscles from forage- (F) and grain-finished (G) beef (10 observations/mean).

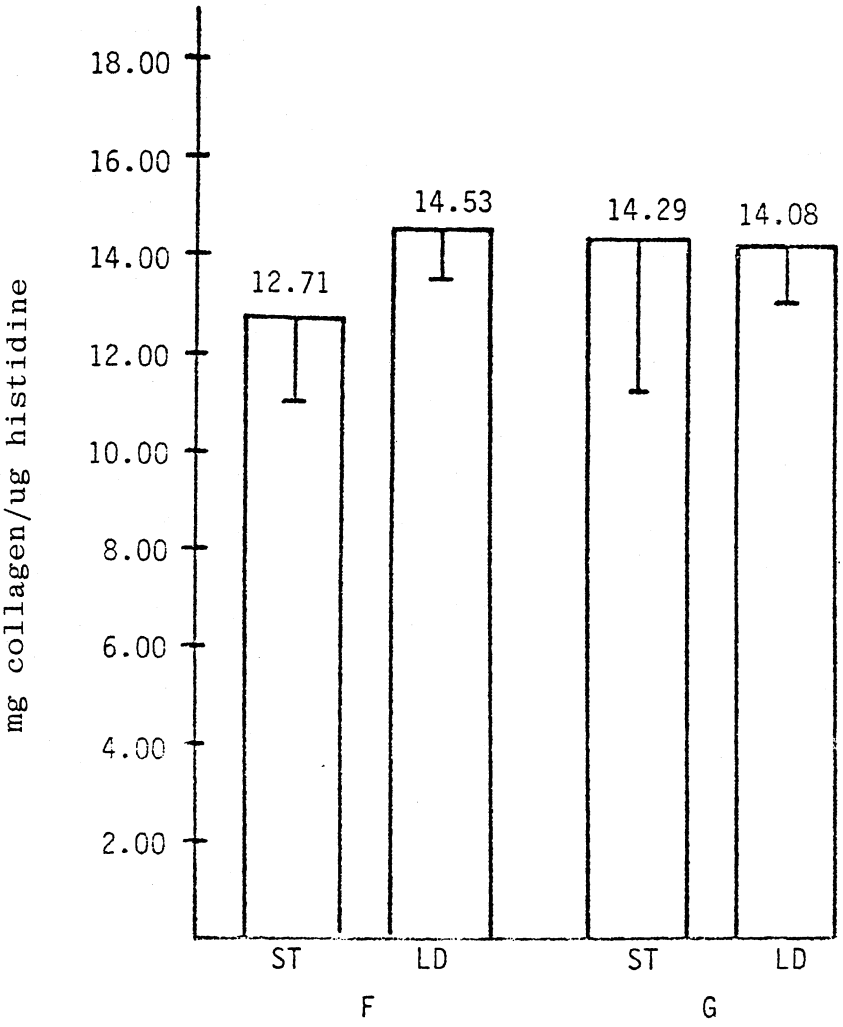


Figure 3. Ratio of insoluble collagen to histidine in longissimus dorsi (LD) and semitendinosus (ST) muscles from forage- (F) and grain-finished (G) beef (10 observations/mean).

the meat would fall apart when it is cooked. However, more research is necessary to determine the optimum number of crosslinks needed in collagen to produce an acceptable product, and to what degree the crosslinks can be altered or manipulated.

The results of this study indicate that intramuscular collagen content or the chemical characteristics of collagen are not the only factors responsible for the variation in tenderness between forage- and grain-finished beef. Further research is necessary to determine if environmental factors possibly play a larger role than collagen in determining the differences between grain- and forage-finished cattle.

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APPENDIX

TABLE VII

INTRAMUSCULAR CONNECTIVE TISSUE EXTRACTION

REAGENTS:

Potassium phosphate buffer (0.1 M):

Mix separately:

27.218 g KH_2PO_4 in 2000 ml H_2O

34.844 g K_2HPO_4 in 2000 ml H_2O

Mix 1610.0 ml of 0.2 M K_2HPO_4 solution with 390.0 ml of 0.1 M KH_2PO_4 solution.

Add 365.222 g KI and adjust to pH 7.4.

Makes 2 liters of 0.1 M potassium phosphate buffer containing 1.1 M potassium iodide, pH 7.4.

Potassium chloride solution:

Mix 149.1 g KCl in 2000 ml H_2O to make 1 M solution.

Sodium chloride solution:

Mix 9.0 g of NaCl in 1000 ml H_2O to make one liter of 0.9% solution.

PROCEDURE:

1. Place 25-g sample in Omni mixer cup.
2. Add 125 ml cold distilled water and blend for 15 seconds, decant liquid through cheesecloth and repeat for 15 seconds.
3. Add 125 ml 0.1 M potassium phosphate buffer, pH 7.4, containing 1.1 M potassium iodide, blend for 15 seconds, decant and repeat for two more 15-second extraction periods.
4. Wash remaining connective tissue with a large volume of 1 M potassium chloride.
5. Wash sample with a large volume of 0.9% sodium chloride.

TABLE VII (Continued)

6. Wash sample with distilled water, place in 50-ml centrifuge tube.

7. Centrifuge at 4000 x g for 5 minutes.

8. Decant supernatant.

Source: Wu et al., 1977

TABLE VIII

AMINO ACID ANALYSIS: SHORT COLUMN

REAGENTS:

Sodium Citrate Buffer:

pH 5.28 ± 0.02
Use short column eluent.

Sodium concentrate 0.35 N		
Sodium citrate · 2 H_2O	1372.6 gm	34.3 gm
Conc. HCl	260 ml	6.5 ml
BRIJ-35 solution 50%	80 ml	2.0 ml
Pentachlorophenol	4 ml	6 drops
Final Volume	40 liters	1 liter

Ninhydrin Reagent:

4 M Sodium acetate	
pH 5.2	150 ml
Dimethylsulfoxide	450 ml
H_2O	300 ml
Ninhydrin	12 gm
Hydrindantin	0.38 gm

Standards: 500 micrograms Type I collagen (Sigma Chem.)
500 micrograms Type II collagen (Sigma Chem.)

PROCEDURE:

1. Place approximately 500 micrograms of lypholyzed crude intramuscular connective tissue in hydrolysis tube.
2. Add 0.2 ml of 6 N HCl to each tube.
3. Evacuate and seal the glass hydrolysis tubes.

TABLE VIII (Continued)

4. Place tubes in 100° C oven for 24 hours.

5. Break open hydrolysis tubes and dry samples in a dessicator under vacuum.

6. Redissolve sample in 0.5 ml sodium citrate buffer.

7. Add a 0.2 ml aliquot to the short column of the amino acid analyzer, using sodium citrate buffer as the running buffer and ninhydrin reagent for color development.

8. Read absorbencies at 480 nm and 540 nm.

TABLE IX

SOLUBLE COLLAGEN ANALYSIS

REAGENTS:

Sodium Phosphate Buffer:

Mix separately:

13.799 g NaH_2PO_4 in 500 ml H_2O
14.196 g Na_2HPO_4 in 500 ml H_2O

Mix 325.5 ml Na_2HPO_4 with 174.5 ml NaH_2PO_4 and adjust to pH 7.0.

Dilute with 500 ml H_2O to make 1 liter of 0.1 M sodium phosphate buffer.

Alkaline Copper Reagent:

Mix just prior to analysis; make fresh every day:

1. Mix 1 ml of 0.05 M $CuSO_4$ with 1.5 ml of 2.5% sodium tartrate.

2. Place 2 ml of copper sulfate-sodium tartrate mixture in a 25-ml volumetric flask and bring to volume with an 8% Na_2CO_3 solution in 0.2 Na OH.

Gelatin Standard:

10,000 ug/ml stock solution--use sodium phosphate buffer, pH 7.0, to dissolve gelatin.

Working solutions of 25,50, 100, 200 and 300 ug/ml were prepared for the standard curve.

PROCEDURE:

1. Weigh duplicate 2-mg samples of lypholyzed intramuscular connective tissue into 15-ml centrifuge tubes.

2. Add 2 ml of 0.1 M sodium phosphate buffer, pH 7.0.

3. Incubate in a water bath at 69° C for one hour.

4. Let cool, then centrifuge at 6000 rpm for 15 min.

TABLE IX (Continued)

5. Place a 1-ml aliquot of sample in a test tube and add 1 ml of alkaline copper reagent and mix immediately.

6. Let stand at room temperature 10 minutes or longer.

7. Add, and immediately mix in, 0.2 ml of dilute (IN) Folin-Ciocalteu reagent.

8. Let stand at room temperature for 45 minutes.

9. Read absorbencies at 750 nm.

Source: Lowery, et al., 1951

TABLE X

PALATABILITY SCORE SHEET

(For Trained Descriptive Attribute Panel)

Judge: _____ Date: _____

INITIAL JUICINESS

8. Extremely juicy
7. Very juicy
6. Moderately juicy
5. Slightly juicy
4. Slightly dry
3. Moderately dry
2. Very dry
1. Extremely dry

SUSTAINED JUICINESS (8 Chews)

8. Extremely juicy
7. Very juicy
6. Moderately juicy
5. Slightly juicy
4. Slightly dry
3. Moderately dry
2. Very dry
1. Extremely dry

TENDERNESS (First Impression)

8. Extremely tender
7. Very tender
6. Moderately tender
5. Slightly tender
4. Slightly tough
3. Moderately tough
2. Very tough
1. Extremely tough

AMOUNT OF CONNECTIVE TISSUE (15 Chews)

8. None
7. Practically none
6. Traces
5. Slight
4. Moderate
3. Slightly abundant
2. Moderately abundant
1. Abundant

BEEF-FLAVOR INTENSITY

8. Extremely intense
7. Very intense
6. Moderately intense
5. Slightly intense
4. Slightly bland
3. Moderately bland
2. Very bland
1. Extremely bland

OFF-FLAVOR INTENSITY

8. None
7. Practically none
6. Traces
5. Slight
4. Moderate
3. Slightly intense
2. Moderately intense
1. Extremely intense

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