

COMPARISON OF PALATABILITY SCORES AND SHEAR VALUES
WITH COLLAGEN NITROGEN VALUES FOR BEEF

by

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INTRODUCTION

Meat has been referred to as the foundation of the American diet, and most consumers have definite ideas on what makes a good piece of meat. Tenderness is one of the most desired qualities. Lean meat consists essentially of muscle fibers, but also it contains considerable and variable amounts of connective tissue. It is reasonable to assume that both of these constituents of muscle have some role in the tenderness of meat.

Chemical, physical, organoleptic, and histological methods have been used to study the characteristics of muscle, and attempts have been made to relate the values obtained for the various measurements to the tenderness of meat. Each of these methods has limitations as criteria for measuring tenderness. Nevertheless, each one can provide information not gained by the others.

Present methods for the chemical analysis of collagen give inconsistent results and are time consuming. The greatest difficulty lies in separating the muscle fiber proteins from the connective tissue proteins. There is some evidence, however, that phosphates may be beneficial in the separation of the soluble proteins from the collagen. Also, it is known that some proteases do not attack collagen. It is possible, therefore, that certain proteolytic enzymes might be employed to disintegrate the complex structure of connective tissue, so that the water soluble proteins may be removed by centrifugation and washing.

It was deemed worthwhile to study the effect on collagen nitrogen values of modifying the blender-centrifuge method that has been used in this laboratory for determining the collagen content of meat. This was done by using proteases and a phosphate wash in the steps required for the separation of soluble proteins from the collagenous material. The objectives of the study reported here were: (1) to attempt to develop a reliable method for using certain enzymes to ascertain the collagen nitrogen in meat, (2) to compare the results of two methods (proteolytic enzyme method and blender-centrifuge method) used for the separation of collagen from the rest of the muscle tissue, as determined by collagen nitrogen values, and (3) to compare tenderness scores and shear values with collagen nitrogen values for the semimembranosus muscle of beef.

REVIEW OF LITERATURE

Nature of Connective Tissue

The proteins of muscle are composed largely of two fractions: (a) the intracellular fraction or the muscle cells; and (b) the extracellular fraction known as connective tissue. The connective tissue within a muscle is composed chiefly of collagenous fibers (mainly collagen) and elastic fibers (mainly elastin) which are embedded in an amorphous ground substance. Lowe (1955) described collagenous fibers as bundles of extremely fine fibrillae, which lie parallel to each other, usually giving a wavy appearance, and offer resistance to a pulling force but lack

elasticity. On the other hand, she stated that elastic fibers are homogeneous, more slender than the collagenous fibers, stretch readily and return to normal length when released.

The collagenous fibers, according to Hiner et al. (1955), are proteinaceous in nature, giving several but not all of the typical protein reactions and are classified as albuminoids. Collagenous tissue dissolves when boiled in water and forms a colloidal solution of gelatin, swells when placed in dilute acids and alkalies, and is difficult to stain. Maximow and Bloom (1952) classify elastin as an albuminoid also, but it is highly resistant to boiling water, acids, and alkalies.

Collagenous fibers may be softened and partially hydrolyzed to gelatin during cooking of meat. Elastic fibers may be softened slightly, but in general, they are affected little by cooking. Hence, the effect of cooking on the tenderness of meat depends upon the balance between the extent of softening the collagenous tissue and the hardening of the muscle fibers through the coagulation of proteins.

Chemical Methods for Determining the Collagen Content of Muscles

Several chemical methods that have been used for assessing the tenderness of meat have attempted to measure the collagen and elastin which are accepted as the main components of connective tissue. In a majority of the methods reviewed the collagen was hydrolyzed to gelatin by means of autoclaving and collagen nitrogen determined by the Kjeldahl method.

Mitchell, Hamilton and Haines Procedure. One of the early methods for extracting and measuring the collagen content of connective tissue was reported by Mitchell et al. (1928). The soluble protein was separated from the connective tissue by placing 100-gram samples of raw ground muscle in ball mills with 300 milliliters of water, grinding with two pounds of balls for 90 minutes, and washing on a 100-mesh sieve. Seven washings, using 100 to 150 milliliters of water at 45° to 50° C. were used to remove practically all soluble proteins. At the same time a good deal of finely divided granular material was lost, which was shown later by Bell et al. (1941) to involve a loss of collagen, and that this loss affected the collagen value obtained by as much as 50 percent.

Bell, Morgan and Dorman Procedure. Bell et al. (1941) modified the "Mitchell" procedure by catching the granular material in a linen filter during the washing process, and by returning this material to the meat residue. The modified method gave better results, but added to the amount of work and time necessary to carry out the analysis. The "Bell" method was used for the determination of collagen in cooked meat with all values being lower for the cooked than for the raw meat. However, the supernatant after autoclaving was not clear, and various methods were used to salt out the colloidal material but none of them proved entirely satisfactory.

Lowry, Gilligan and Katersky Procedure. Lowry et al. (1941) developed a method for extracting the soluble proteins with 0.1 normal sodium hydroxide. A specially prepared sample was weighed

before and after autoclaving, and the grams of collagen were expressed as percent by weight of the total weight. The method described was considered to be more specific than other available methods.

Hartley and Hall Procedure. Hartley and Hall (1949) observed that a non-turbid aqueous extract of raw meat could be obtained by centrifugation of aqueous dispersions of beef muscle tissue adjusted to the isoelectric point (pH 5.07). Therefore, they substituted a rapid centrifugation operation for the sieve and linen filter procedure of the "Bell" method. Also, the ball-milling step was replaced by homogenization in the Waring Blendor. This method was found to give reproducible results when applied to raw beef, but it was not as precise when applied to cooked beef.

In work done by Griswold and Leffler (1952) it was found that the method of Lowry with slight modifications was superior to that of Hartley and Hall, which gave higher values for cooked than for raw meat. Because these two methods agreed within five percent when using purified steer-hide collagen it was believed that in the Hartley-Hall method all of the soluble proteins were not removed and that they affected the collagen nitrogen value.

Miller and Kastelic Modifications. Miller and Kastelic (1956) reviewed the procedures that have been used for partitioning muscle and measuring the connective tissue fraction. They reported exploratory experiments in which they used several methods for separating collagen from other muscle proteins, and in which they calculated collagen nitrogen from the hydroxyproline

content of the sample as well as determining nitrogen values by the micro-Kjeldahl method. Efforts were made to account quantitatively for the total nitrogen content of the tissue.

These authors found that the use of the silent cutter for macerating the muscle tissue resulted in the most uniform sampling and in the most nearly uniform nitrogen values. Since they could not successfully separate the soluble protein fraction from the stroma proteins by centrifugation, they devised, as a separatory technique, a method for the entrapment of the stroma proteins on a mass of glass wool in an Erlenmeyer flask.

Papain was used by Miller and Kastelic (1956) for the fractionation of connective tissue. They found that measurable amounts of protein were solubilized by papain digestion, but neither collagen nor elastin fibers had been solubilized at either an acid or an alkaline pH. This is in agreement with the report of Wang and Maynard (1955) who found that papain (Adolph's meat tenderizer) and Rhozyme P-11 (Rohm and Haas Company) attacked muscle fiber protein (the nuclei of muscle fibers and cells located in the endomysia), but was inactive to collagenous and elastic fibers at room temperature.

It was pointed out by Miller and Kastelic (1956) that alkali has been widely used as an extractant for soluble proteins in fractionation processes, followed by autoclaving the residue, presumably collagen, for the conversion of collagen to gelatin. They stated that the use of these techniques has been based upon the assumption that the collagen has not been affected by prior treatment of the tissue, that the autoclaving selectively

hydrolyzed the collagen to gelatin and that a subsequent determination of nitrogen in the substrate was a valid estimate of the collagen content. However, it was found by various workers, using rat tail tendon and hide powder instead of muscle tissue, that collagen can be dissolved by dilute alkaline solutions. Miller and Kastelic (1956) compared several extracting solutions of differing molarities for the efficiency of protein extraction. Because a mild extractant was desired, a solution of 0.6 M potassium chloride was used along with varying strengths of sodium hydroxide. Estimates of collagen and elastin content of autoclaved potassium-chloride-insoluble muscle tissue based on Kjeldahl nitrogen values were much higher than the collagen or elastin content determined by the hydroxyproline procedure if 0.1 N sodium hydroxide was not used on the residue after the potassium chloride extraction.

Miller and Kastelic (1956) stated that the procedures now in use for studying the tenderness of muscle neglect to take into consideration the ground substance in which collagenous and elastic fibers are embedded, and that this may play an important part in determining the tenderness of meat.

Physical, Organoleptic and Histological Methods for Studying Tenderness

Physical Methods. One of the most common physical methods used to measure tenderness is the mechanical device known as the Warner-Bratzler shearing apparatus. This apparatus measures the number of pounds of force required to shear a cylinder of meat of

a given diameter. Paul and Bratzler (1955) found close agreement between shear values for one-half and one-inch cylinders of meat, and suggested that either size cylinder may be used to measure "shear-tenderness."

Other mechanical devices as listed by Lowe (1955) for estimating tenderness include gauges and penetrometers which appear to measure the density and compactness of muscle. Proctor et al. (1956) developed a denture tenderometer which simulates the dental surfaces and chewing motions in the mouth.

Organoleptic Methods. Taste panels are used to evaluate the tenderness of meat by giving it a numerical score within a given range such as from 10 to one or from seven to one. A method designed to help the panel standardize the procedure of scoring beef for tenderness was reported by Harrison et al. (1949). In this method the scorer counted and recorded the number of chews to masticate a standard sample to a determined end point. The tenderness scores given were then based upon a predetermined number of chews. Each panel member worked out his own system for scoring according to the number of chews required per sample. The procedure of counting chews was used by Paul et al. (1956) who found a highly significant correlation between chews and tenderness scores.

Harrison et al. (1949) found that shear force values (pounds to shear a one-inch cylinder) followed the same pattern as the tenderness scores for five beef muscles. Mackintosh et al (1936), Ramsbottom and Strandine (1948) and Griswold (1955) obtained highly significant correlations between shear and tenderness

values for beef, but Deatherage and Garnatz (1952) reported that although there appeared to be a relationship between tenderness and shear values for shortloin broiled steaks, correlation coefficients for their data were not significant.

Histological Studies. Microscopic studies of the connective tissues in beef muscle have been reported by several workers, and the findings related to the tenderness of muscle. In several of the studies the relationship between the histological evaluation of tenderness and tenderness scores and/or shear force values were pointed out. Some of these reports will be reviewed here.

Hiner et al. (1955) placed muscles in four tenderness groups after making observations of fiber diameter and the amount of connective tissue appearing in photomicrographs of sections prepared from the muscles. According to their observations, the number of collagenous and elastic fibers present affected the tenderness of muscle. The numerical histological ratings for the quantity of collagenous and elastic tissues in certain beef muscles presented by Harrison et al. (1949) agreed with the chemical determinations for the quantity of collagen and elastin in the same muscles. In general, the most tender beef, as measured by tenderness scores and shear values, came from the muscles having the least connective tissue.

A significant correlation coefficient ($r = 0.6$) for the shear values and histological ratings of cooked beef was reported by Ramsbottom and Strandine (1948). On the other hand, Paul et al. (1956) did not obtain a significant correlation coefficient for the numerical ratings given to the amount of collagen and

elastin in raw beef and the tenderness scores for cooked meat, nor for measurements of the width of collagen fibers (in relation to the width of the sections studied) and tenderness scores. Although the correlation coefficients were not significant there was some relationship between these factors for raw beef and the tenderness scores for cooked meat.

Paul et al. (1956) also noted that the collagen in cooked samples accepted Weigert's triple stain in different degrees after various amounts of cooking. Therefore, they suggested the possibility of following the heat-induced alteration of collagenous tissue by staining.

EXPERIMENTAL PROCEDURE

Meat Used

Ten top rounds graded U. S. Good were obtained from a local wholesale meat firm. The rounds were approximately 12 pounds each, and had similar ripening periods. The proximal ends of the rounds were trimmed at the wholesale house to give an even surface. Four 1.5-inch slices were cut across each round, starting at the proximal end, and wrapped in one package. Upon delivery, each slice was wrapped in aluminum foil, labeled as to round and slice number (starting at the proximal end of round number I) and stored at -20° F. until used in the experiment.

Design of the Experiment

The experiment was designed so that four slices, all from one round, were prepared at each cooking period. The slices from

each round were divided into two blocks of two slices each, and one slice from each block was selected at random to be cooked for 30 minutes and the other slice for 45 minutes. The specific design is given in Table 1.

Table 1. Cooking time, in minutes, for 40 slices from ten top rounds.

Round number	: Slice number	: Time in minutes	: Round number	: Slice number	: Time in minutes
I	1	45	VI	21	30
	2	30		22	45
	3	30		23	45
	4	45		24	30
II	5	30	VII	25	45
	6	45		26	30
	7	45		27	30
	8	30		28	45
III	9	45	VIII	29	45
	10	30		30	30
	11	45		31	30
	12	30		32	45
IV	13	45	IX	33	30
	14	30		34	45
	15	45		35	30
	16	30		36	45
V	17	30	X	37	30
	18	45		38	45
	19	45		39	30
	20	30		40	45

A grid, prepared to conform with the shape of the slices of round, was divided into one-inch squares (Plate I). The squares were numbered and a table of random numbers was used to select the numbers on the grid that represented the position in each slice to be used for palatability and shearing tests.

EXPLANATION OF PLATE I

A diagram of the numbered grid composed of 1-inch squares used to select the portions of each steak used for palatability and shearing tests.

PLATE I



Cooking, Palatability and Shearing Tests

Prior to cooking, the slices were allowed to thaw for 48 hours in a refrigerator (40° F.). Six cores for determining raw shear values were removed from each slice and the slice weighed. The slice was then placed on a rack in an electric pressure cooker with one-fourth cup water and cooked under 10 pounds pressure for either 30 or 45 minutes. Only two cookers were available; therefore, two slices were cooked individually for 45 minutes, and then wrapped in aluminum foil while the other two slices were cooked for 30 minutes.

The pressure regulator was lifted at the end of the cooking period to allow the pressure to go down immediately. The slices were weighed, six cores removed for shear values, and six samples 1/6-inch thick removed for palatability tests. A panel of six members rated each slice for aroma, flavor, juiciness and tenderness on a 10 to one point scale, with 10 as the highest score. Form I, Appendix, is the score card that was used.

Both raw and cooked cores were sheared on a Warner-Bratzler shearing apparatus, and an average of the values obtained from four shears was considered the shear value for each core. The cores were then ground twice through a Universal hand grinder using a fine blade, packed into four-ounce screw cap jars and stored at -20° F. to be used later for chemical analyses.

Chemical Analyses

Preliminary Work. While working with the Hartley and Hall (1949) method, Hall (1955) noted that the supernatants obtained

during a series of washings after the blending process became increasingly cloudy after each washing. This condition indicated that some collagenous material might be lost in the dispersion. Various salts (NaCl , MgCl_2 , CaCl_2) were added to the wash water, but they did not clear the supernatant liquid. Also, adjustment of pH did not seem to help, so it was decided that the colloidal tendency could not be attributed to adjustment to a false isoelectric point. Since the main cloudiness did not appear until the fourth or fifth washing, it was believed that perhaps the water wash decreased concentration of water soluble natural components of meat which had originally prevented dispersion. Therefore, a phosphate wash, calculated to be equal to the molarity of the phosphate in the meat (0.06 M, pH 5) was used, and seemed to prevent the colloidal dispersion. This is in agreement with the work of Mohler and Kiermeier (1953) who found that metaphosphates even in small concentrations and at the prevailing pH of the meat caused flocculation of the meat albumin. These authors found an increase in sediment volume produced on mixing finely ground meat in water when phosphate salts were added. This increase in volume was not related to pH for it was greater than that produced by using sodium hydroxide instead of phosphate to adjust to the same pH values. The volume increase was said to be due to increase in cohesion, thus increasing hydration. This interpretation might be open to question, since an increase in hydration would be expected to involve an increase in dispersion. It seems more likely that the cohesive function of the phosphate involves a bridge bond between portions joined in the large aggregates.

Three enzymes, Protease 15, Rhozyme P-11 and Rhozyme A-4 were obtained from the Rohm and Haas Company, and were used to separate the soluble proteins from collagen in muscle. The P-11 and A-4 gave varied and inconsistent results and produced a colloidal dispersion during the washing process. However, the Protease 15 did not act in this manner and was used in an attempt to develop an improved method for determining collagen nitrogen.

When the Hartley-Hall blender-centrifuge procedure was modified to use the phosphate wash instead of water for the homogenate, and the NaCl wash instead of water for the autoclaved material, clear supernatants and clean pour-offs persisted through the series of washings. These modifications were accompanied by better agreement between duplicate determinations, and were used in this investigation to compare collagen nitrogen values obtained by enzyme and by blender dispersion.

Collagen Nitrogen by Enzyme Method. The ground meat was removed from the freezer and allowed to thaw in a refrigerator overnight. After mixing thoroughly in the jars, duplicate 10-gram samples of meat were weighed into four-ounce screw cap bottles. Forty milliliters of water and 0.1 gram of enzyme (Protease 15)¹ were added to each sample along with enough 0.75 N sodium hydroxide to bring the mixture to pH 7.35 and the sample was mixed for one minute with a motor stirrer. The mixture was adjusted to pH 7.35, because according to literature supplied by the company which furnished the enzyme, Protease 15 reacts best at this pH.

¹ Trade name of the protease enzyme supplied for this work by Rohm and Haas, Philadelphia.

In order to determine the amount of sodium hydroxide necessary to adjust the pH of the meat suspension, the pH of a blended sample was determined with a glass electrode potentiometer. A tentative amount (1 ml) of the base was mixed with the meat and water. The pH was again determined, and by proportion, the theoretical amount of base was calculated, added to the meat and mixed. The total amount of base necessary to adjust the sample was added to the duplicate sample at one time, and mixed only once. Toluene (1 ml) was added to each sample as a preservative.

The samples were then placed in a shaker in a water bath which was maintained at a constant temperature of 40° C. for 16 hours. At the end of this period the samples were cooled to 25° C. by placing the bottles in cold water. They were adjusted to pH 5.0 by the addition of 1 N sulfuric acid in the manner described above. After adjustment the homogenate was rinsed with 0.06 M phosphate wash (45° to 50° C.) into a 250-milliliter beaker. The liquid was decanted into 40-milliliter centrifuge tubes, centrifuged for three minutes at 2600 r.p.m. and the supernatant discarded. The remaining digest was rinsed into the tubes and centrifuged. Approximately 25 milliliters of the phosphate wash solution was added to the precipitate, stirred thoroughly and centrifuged. The samples were washed in this manner five times. In the preliminary work it was found that the Biuret test was negative on the supernatant from the fifth washing, therefore indicating that all water soluble proteins were washed out and would not affect the collagen nitrogen value.

The residue was rinsed with water into 250-milliliter beakers and the volume brought up to approximately 80 milliliters. Watch glasses were placed over the beakers and the beakers placed in an autoclave for two hours at 15 pounds pressure. At the end of this period the samples were removed from the autoclave and the supernatant was decanted, while warm, through filters into Kjeldahl flasks. The precipitate was then transferred to centrifuge tubes and washed five times with 0.2 percent sodium chloride solution (boiling). Each time the supernatant was filtered into the Kjeldahl flasks. The precipitate was then discarded, and after allowing the filters to drain completely, 25 milliliters of concentrated sulfuric acid were added to each flask and the flask placed in a rack to be analyzed later for collagen nitrogen content. Total nitrogen was determined on samples of approximately two grams of the original material and collagen nitrogen was expressed as percent of total nitrogen.

During the phosphate wash-centrifuging process it was noted that the supernatant from some of the samples was slightly colloidal. This indicated that collagen might be discarded or that some of the soluble protein might not be removed during the centrifuging and subsequent decanting process. Therefore, several adjustments in the procedure were made in an attempt to overcome this situation. An increase in the centrifuge speed and time helped, but did not overcome the difficulty. Also it was noted that the supernatant from the raw samples tended to settle on standing. Therefore, one run of samples was set into the refrigerator for 24 hours after adjusting the pH to 5.0. This gave

some aid in precipitating the colloidal material, but not enough to be worth the time required. Since it was found in earlier work that the phosphate wash helped to precipitate a colloidal solution, 0.4 gram of phosphate in the form of KH_2PO_4 was added to each sample after incubation and the mixture placed in the refrigerator for 24 hours. This treatment did not overcome the colloidal condition.

Next, the supernatant from one run of four raw samples was saved, allowed to settle and decanted. The precipitate was then treated as a regular sample, centrifuged and autoclaved. The results were varied from sample to sample, and it was believed that the value obtained for collagen nitrogen was so small that the collagen nitrogen values for regular samples would not be affected by the occurrence of colloidal material in the supernatant.

Moreover, while working with the samples it was noted that all samples did not behave in a like manner. The supernatant from those in which the base and acid were added with two mixings was more colloidal than that from the duplicate samples in which there was only one mixing. Therefore, it was decided to add the base and acid to all samples in a like manner, i.e., by dropping from a pipette directly into the sample while mixing, and using the two mixings as necessary for proper adjustment of pH. However, in some cases colloidal conditions persisted in the supernatant. In various attempts to overcome this condition, chloroform was added along with toluene as a preservative during incubation. Non-colloidal supernatants resulted, but became slightly cloudy in fourth and fifth washings if water was used as a wash.

With the phosphate wash, clear supernatants persisted to the end of the fifth washing.

The change in the method for adding the acid and base and the addition of chloroform overcame the difficulty of a colloidal suspension in the supernatant, and were used for the last 14 samples. The revised procedure was as follows: Weigh the sample, add enzyme, water and check pH. Add one milliliter of base while mixing, take pH and calculate the additional base necessary and add while mixing. Add one milliliter each of toluene and chloroform, and place the samples in a shaker in a water bath at 40° C. overnight. Remove samples, cool, take pH, add one milliliter acid while mixing, check pH and calculate the additional acid necessary to reach pH 5 and add while mixing. Transfer the samples to centrifuge tubes and wash by centrifugation with the phosphate solution at 3000 r.p.m. for five minutes. Repeat five times. Transfer the precipitate to beakers, fill to approximately 80 milliliters with water and autoclave.

Decant the warm supernatant through filters into Kjeldahl flasks. Transfer the precipitate to centrifuge tubes and wash by centrifugation with boiling 0.2 percent sodium chloride solution. Filter each supernatant into Kjeldahl flasks, and discard the precipitate after five washes. Add 25 milliliters concentrated sulfuric acid to the Kjeldahl flasks and place in the rack to be analyzed for collagen nitrogen at a later date. The collagen nitrogen usually was determined within a week by the Kjeldahl-Wilfarth-Gunning method (A.O.A.C. 2.24).

Collagen Nitrogen by Blendor-Centrifuge Method. Aliquots of the same ground meat used for determining collagen nitrogen by the enzyme method also were analyzed by a blendor-centrifuge method; and the collagen nitrogen values obtained by the two methods were compared. The Hartley-Hall (1949) method was followed with two exceptions. Phosphate wash was used instead of water as a wash before autoclaving and 0.2 percent sodium chloride solution was used for washing after autoclaving.

Total Nitrogen. The analysis for total nitrogen was carried out by the following procedure. Samples of one and one-half to two grams of finely ground meat were weighed by difference from a tall weighing bottle containing a plastic spoon for transferring the sample. The sample was transferred to a filter paper, which was then rolled up and dropped into a Kjeldahl flask. Concentrated sulfuric acid (37.5 ml) was added and the nitrogen determined by the Kjeldahl-Wilfarth-Gunning method (A.O.A.C. 2.24).

Statistical Analysis

Correlation coefficients for raw meat and meat cooked 30 and 45 minutes were obtained for the following: (1) collagen nitrogen values obtained by the enzyme method vs. shear values, and (2) collagen nitrogen values obtained by the blendor-centrifuge method vs. shear values. Also, correlation coefficients for meat cooked 30 minutes and 45 minutes were computed for: (1) collagen nitrogen values by the enzyme method vs. tenderness scores, (2) collagen nitrogen values by the blendor-centrifuge method vs. tenderness scores, (3) shear values for cooked meat vs. tenderness

scores, and (4) shear values for cooked meat vs. shear values for raw meat.

The t-test was used on certain data to compare the following: (1) collagen nitrogen values as determined by the enzyme method vs. collagen nitrogen values determined by the blender-centrifuge method for both raw and cooked meat, and (2) collagen nitrogen values for samples cooked 30 minutes vs. the values for samples cooked 45 minutes for both the enzyme and blender-centrifuge methods of determining collagen.

RESULTS AND DISCUSSION

A palatability committee scored the cooked steaks for aroma, flavor, juiciness and tenderness. In addition, shear values and collagen nitrogen values were determined for both raw and cooked samples from each steak. Averages of mean values for these tests are included in tables in the body of the thesis, whereas the more detailed data are in the Appendix.

Aroma, Flavor and Juiciness

The aroma and flavor scores (Table 2) varied slightly from round to round, but the average of mean scores indicated that there were no noticeable differences which could be attributed to the cooking times used in this study. With both the 30 and 45 minute cooking periods the steaks were well-done, hence the low juiciness scores were expected. Average of mean scores for this factor varied more from round to round than those for aroma and flavor; however, there was little difference between the average

Table 2. Averages of mean aroma, flavor, and juiciness scores¹ for steaks cooked 30 minutes and 45 minutes.

Round number	Cooking time					
	30 minutes			45 minutes		
	Aroma	Flavor	Juiciness	Aroma	Flavor	Juiciness
I	8.2	6.7	4.2	8.3	6.9	4.2
II	8.0	7.1	3.6	8.0	7.0	3.3
III	8.5	7.0	2.9	8.4	7.5	2.7
IV	8.3	7.4	3.2	8.4	7.7	3.4
V	8.4	7.5	3.7	8.4	7.4	2.7
VI	8.5	7.4	3.9	8.4	7.0	3.1
VII	7.5	6.4	3.7	8.1	7.4	4.1
VIII	8.0	7.0	3.7	8.3	7.3	3.1
IX	8.1	7.5	4.3	8.1	7.4	3.4
X	8.3	7.6	3.3	8.3	7.7	3.1
Average	8.2	7.2	3.7	8.3	7.3	3.3

¹ Maximum score possible, 10.

of mean scores for the steaks cooked for 30 and 45 minutes (Table 2).

Tenderness

Shear Values for Raw and Cooked Meat. Averages of mean values for tenderness as measured by shear values and palatability scores are given in Table 3. For all rounds the average shear value for meat cooked either 30 or 45 minutes was less than that for the raw meat. A non-significant correlation coefficient

Table 3. Averages of mean ratings for tenderness by shear values and palatability scores.¹

Round number	Shear values, lbs.			Tenderness scores	
	Raw	30'	45'	30'	45'
I	25.8	20.6	12.5	8.0	8.7
II	19.4	16.4	11.0	8.1	9.0
III	22.9	19.1	15.3	7.7	8.5
IV	20.5	11.3	10.9	6.4	7.8
V	27.5	11.6	14.4	7.5	8.2
VI	27.3	16.1	16.0	7.3	7.9
VII	27.5	16.4	11.9	7.6	7.8
VIII	24.6	21.4	17.0	6.6	8.4
IX	24.4	22.2	22.2	7.2	6.4
X	26.2	17.3	17.4	7.0	7.9
Average	24.6	17.2	14.9	7.3	8.1

¹ Maximum score possible, 10.

($r = 0.32$, Table 4) was obtained for the shear values for meat cooked 30 minutes and the shear values for the raw meat. On the other hand, a significant ($P < .05$) correlation coefficient ($r = 0.49$) was obtained when comparing the shear values for meat cooked 45 minutes and those for raw meat. This indicates that there was no relationship between shear values for raw meat and those for meat cooked 30 minutes, but that there was a slight relationship between shear values for raw meat and for meat cooked 45 minutes.

Table 4. Correlation coefficients for certain measurements made on the steaks from ten top rounds.

Measurements correlated	Correlation coefficient and significance	Conclusions
Raw shear values vs. cooked shear values		
30 minutes	0.32 ns	No relationship
45 minutes	0.49 *	Slight relationship
Shear values vs. tenderness scores		
30 minutes	-0.04 ns	No relationship
45 minutes	-0.65 **	Tenderness scores related to shear values
Collagen nitrogen values vs. tenderness scores		
Enzyme method		
30 minutes	-0.44 *	Slight relationship
45 minutes	-0.51 *	Slight relationship
Blendor-centrifuge		
30 minutes	0.04 ns	No relationship
45 minutes	-0.28 ns	No relationship

ns - non-significant

* - significant ($P < .05$)

** - highly significant ($P < .01$)

Shear Values and Tenderness Scores. In general, averages of mean tenderness scores for the rounds cooked for 30 minutes were slightly lower and the shear values higher than those for meat cooked 45 minutes (Table 3). A non-significant correlation coefficient of -0.04 indicated that there was no relationship between shear values and tenderness scores for meat cooked 30 minutes (Table 4). However, a highly significant correlation coefficient of -0.65 pointed out that for meat cooked 45 minutes the tenderness scores were definitely related to the shear values

(Table 4). This inverse relationship or negative correlation coefficient is to be expected because a high tenderness score and a low shear value indicate tender meat. Also, Hood et al. (1955) found a significant negative correlation coefficient ($r = -0.52$) for shear force and tenderness scores. Moreover, the correlation coefficients were significant whether the samples were cooked by dry or moist heat. Likewise, Griswold (1955) reported a highly significant correlation ($r = -0.58$) between shear force and tenderness scores when data for 118 paired observations were analyzed. On the other hand, Deatherage and Garnatz (1952) tested processed meat and reported a relationship between tenderness scores and shear values, but the correlation coefficient was not significant.

Shear Values and Collagen Nitrogen Values. Average of mean values for shear force are given in Table 3 and those for collagen nitrogen are given in Table 5. Correlation coefficients for these data are presented in Table 6. For raw meat there was no discernable relationship between shear values and collagen nitrogen values as measured by both the enzyme and blender-centrifuge methods. However, the correlation coefficients for shear values and collagen nitrogen values (enzyme method) were near significant ($r = 0.39$) and very highly significant ($r = 0.68$) for meat cooked 30 and 45 minutes, respectively. On the other hand, no relationship was found between these factors when the collagen nitrogen values were measured by the blender-centrifuge method. This was true for meat cooked both 30 and 45 minutes.

Table 5. Averages of mean collagen nitrogen values¹ determined by the enzyme and blender-centrifuge methods.

Round number	Enzyme method			Blendor-centrifuge method		
	Raw	Cooked		Raw	Cooked	
		30'	45'		30'	45'
I	7.6	7.2	6.7	9.9	9.5	9.2
II	8.5	6.1	5.9	10.2	9.5	8.5
III	8.9	6.5	6.3	10.6	9.7	8.6
IV	8.8	6.2	6.5	10.0	8.7	8.6
V	10.4	6.9	6.5	11.1	9.2	8.7
VI	9.2	6.5	6.7	9.8	9.3	9.3
VII	9.2	7.3	7.4	11.1	9.8	8.9
VIII	10.2	9.9	9.6	10.8	10.1	9.3
IX	11.1	10.0	9.9	11.1	9.6	9.6
X	11.8	10.3	9.9	10.4	9.9	9.1
Average	9.6	7.7	7.5	10.5	9.5	9.0

¹ Expressed as percent of total nitrogen.

In general, the average shear value for the samples cooked 30 minutes was greater than that for the meat cooked 45 minutes (Table 3). This indicated that usually the longer cooking period made the meat more tender; therefore, presumably it would contain less collagen. In line with this finding, collagen nitrogen values measured by the blender-centrifuge method, were significantly ($P < .001$) greater for the samples cooked 30 minutes than for those cooked 45 minutes (Table 7). This resembles the work of Griswold (1955) whose data indicated that (although the

Table 6. Correlation coefficients for collagen nitrogen values determined by the enzyme and blender-centrifuge methods and shear values.

Method for collagen determination	Variable	Correlation coefficient and significance	Conclusions
Enzyme	Raw	0.10 ns	No relationship
Blendor-centrifuge	Raw	0.22 ns	No relationship
Enzyme	Cooked 30' 45'	0.39 near * 0.68 ***	May be weak relationship Collagen nitrogen values related to shear values
Blendor-centrifuge	Cooked 30' 45'	0.21 ns 0.28 ns	No relationship No relationship

ns - non-significant

* - significant ($P < .05$)

*** - very highly significant ($P < .001$)

Table 7. Comparison of collagen nitrogen values obtained by enzyme and blender-centrifuge methods by the t-test.

Collagen nitrogen values:	t value and significance	Conclusions
30 minute vs. 45 minute		
Enzyme method	0.37, 38 df, ns	No cooking time effect
Blendor-centrifuge method	3.33, 38 df, ***	Collagen nitrogen greater for 30' cooking than for 45' (very low error variance)
Enzyme vs. Blendor-centrifuge method		
Raw	4.50, 39 df, ***)	Collagen nitrogen
Cooked 30'	5.20, 19 df, ***)	higher with blender-
Cooked 45'	4.12, 19 df, ***)	centrifuge method.

df - degrees of freedom

*** - very highly significant ($P < .001$)

differences were not significant) there was a decrease in the collagen as the internal temperature of the meat increased.

However, when the collagen nitrogen was determined by the enzyme method, there were no significant differences between the values obtained for meat cooked for the two periods used in this study (Table 7). This indicated that the cooking time did not affect the collagen nitrogen values, and seemingly there was no decrease in collagen that was attributable to extended cooking.

Collagen Nitrogen Values and Tenderness Scores. Correlation coefficients for collagen nitrogen values and tenderness scores are given in Table 4. There was a significant ($P < .05$) relationship between the tenderness scores and collagen nitrogen values (enzyme method) for meat cooked 30 minutes ($r = -0.44$) and for that cooked 45 minutes ($r = -0.50$). On the other hand, when the collagen nitrogen values were determined by the blender-centrifuge method there was no relationship with the tenderness scores for meat cooked either 30 or 45 minutes. Cover et al. (1956) reported results from studies using moist and dry heat methods for cooking beef. Their data indicated that the collagen content of beef was associated with tenderness when the same muscle was cooked by different methods. However, when the tenderness of the two muscles cooked by the same method was compared, the collagen content of the muscles was not associated with tenderness.

Enzyme Method vs. Blender-Centrifuge Method. In general, with both raw and cooked beef, collagen nitrogen values averaged higher under the blender-centrifuge method than under the enzyme method (Table 5). When the data from all 40 samples were

subjected to the t-test, the collagen nitrogen values were significantly ($P < .001$) higher when determined by the blender-centrifuge method than when measured by the enzyme method (Table 7). However, because of difficulty in decanting the supernatant from samples treated with the enzyme, this method was changed (page 18) to avoid a colloidal dispersion in the supernatant. After the change was made, higher values were obtained for collagen nitrogen. This might have indicated inhibition of the enzyme action by the addition of chloroform. In a supplemental experiment chloroform was added to duplicate samples both before and after incubation. It was equally successful in flocculating the colloidal material when added either before or after incubation. Therefore, the effect of chloroform appeared to be mainly mechanical, i. e., by adsorption on colloidal particles, cementing them together and increasing their specific gravity.

Chloroform was not added to the first 24 samples in which collagen nitrogen was determined by the enzyme method. Therefore, the data for these samples were analyzed separately from the data from the last 16 samples. The t-test indicated that for the first 24 samples the collagen nitrogen values determined by the blender-centrifuge method were significantly ($P < .001$) higher than those obtained by the enzyme method (Table 8). On the other hand, there were no differences noted between the values obtained by the two methods after the change in procedure was made.

Throughout the experiment there was better relationship between shear values for raw and cooked meat, shear values and tenderness scores and shear values and collagen nitrogen values

Table 8. Comparison of enzyme and blender-centrifuge methods for determining collagen nitrogen by the t-test.

Enzyme vs. blender-centrifuge method	t value and significance	Conclusions
Samples		
1-24, raw	5.38, 46 df, ***	Collagen nitrogen values greater by the blender-centrifuge than by the enzyme method.
1-24, 30 min.	12.73, 22 df, ***	
1-24, 45 min.	9.60, 22 df, ***	
25-40, raw	0.64, 30 df, ns	Collagen nitrogen values approximately equal by the two methods.
25-40, 30 min.	0.77, 14 df, ns	
25-40, 45 min.	0.00, 14 df, ns	

df - degrees of freedom
 *** - very highly significant ($P < .001$)
 ns - non-significant

(enzyme method) when these factors were compared for meat that had been cooked for 45 minutes than for that cooked 30 minutes. Also, better relationships between collagen nitrogen values and the factors listed above were found when the enzyme method rather than the blender-centrifuge method was used for the collagen nitrogen determination.

Total Nitrogen

All samples of raw and cooked meat were analyzed for total nitrogen so that collagen nitrogen could be expressed as percent of total nitrogen. Both total nitrogen and collagen nitrogen were reported on the wet basis. There was little variation among the rounds in the percent of total nitrogen. The mean values (Table 14, Appendix) for the total nitrogen in all raw samples

ranged from 3.58 to 4.11 and averaged 3.81 percent. The percent total nitrogen in the meat cooked for 30 minutes ranged from 5.84 to 6.52 with an average of 6.26. When the meat was cooked for 45 minutes the percent total nitrogen ranged from 5.95 to 6.69 with an average of 6.37. This is in line with work reported by Harrison et al. (1953) who stated that evaporation of moisture during cooking resulted in a larger percent of total nitrogen in the cooked than in the raw tissue. They also noted that values were lower for meat cooked to a lower internal temperature because there was less evaporation of moisture.

SUMMARY

Forty paired steaks from ten top rounds, graded U. S. Good, were cooked in a pressure cooker (10 p.s.i.g.) for 30 and 45 minutes. They were compared for aroma, flavor, juiciness, tenderness, shear force and collagen nitrogen content as determined by the enzyme and the blender-centrifuge methods.

Aroma, flavor, juiciness and tenderness were rated on a 10 to one scale by a palatability panel of six members. There were no noticeable differences in aroma, flavor and juiciness scores which could be attributed to the cooking times used in this study. However, the steaks that were cooked 30 minutes were rated slightly less tender by the panel than those that were cooked 45 minutes.

For all rounds the average shear value for the cooked meat was less than that for the raw meat. There was no relationship between shear values for raw meat and meat cooked 30 minutes, but

there was a slight relationship between shear values for raw meat and those for meat cooked 45 minutes.

Similar to the shear values for raw meat and that cooked for 30 minutes, there was no relationship between shear values and tenderness scores for the meat cooked 30 minutes. On the other hand, the tenderness scores were definitely related to shear values for meat cooked 45 minutes.

A near significant correlation coefficient was obtained for shear values and collagen nitrogen values determined by the enzyme method for meat cooked 30 minutes, and a highly significant coefficient when the meat was cooked 45 minutes. However, there was no relationship between shear values and collagen nitrogen values for meat cooked for either 30 or 45 minutes when the collagen nitrogen values were determined by the blender-centrifuge method. Moreover, there was no relationship between shear values and collagen nitrogen values for raw meat determined by either method.

Similar to the relationship between shear values and collagen nitrogen values obtained by the enzyme method, tenderness scores and collagen nitrogen values determined by the enzyme method were significantly related for samples cooked both 30 and 45 minutes. Again, there was no relationship when the same factors were compared using the blender-centrifuge method for determination of collagen nitrogen.

When the data for all samples were pooled for statistical analysis, collagen nitrogen values were significantly ($P < .001$) higher for both raw and cooked beef when determined by the

blendor-centrifuge method than when determined by the enzyme method. A change was made in the enzyme method after the first 24 samples were run (chloroform was added before incubation); thus, these data were analyzed separately from the last 16 samples. Under these circumstances, the t-test indicated that the collagen nitrogen values for the first 24 samples were significantly ($P < .001$) higher when measured by the blendor-centrifuge method than when determined by the enzyme method, but for the last 16 samples there were no significant differences between the collagen nitrogen values obtained by the two methods.

In this experiment, there was higher correlation between the factors compared when the meat was cooked for 45 minutes than when it was cooked 30 minutes. Also, better relationships were found when the enzyme method was used for collagen nitrogen determination than the blendor-centrifuge method.

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APPENDIX

Form I.

SCORE CARD FOR MEAT

Judge	Sample No.										Kind	Date
FACTOR	1	2	3	4	5	6	7	8	9	10		
Aroma	: :Extremely: :Good	: :Very :good	: :Good	: :Plus: :	: :Medium: :	: :Minus: :	: :Fair: :	: :Poor :	: :Very :poor	: :Extremely: :poor	: :	: :
Flavor	: :Extremely: :good	: :Very :good	: :Good	: :Plus: :	: :Medium: :	: :Minus: :	: :Fair: :	: :Poor :	: :Very :poor	: :Extremely: :poor	: :	: :
Juiciness	: :Extremely: :juicy	: :Very :juicy	: :Juicy	: :Plus: :	: :Medium: :	: :Minus: :	: :Fair: :	: :Dry :	: :Very :dry	: :Extremely: :dry	: :	: :
Tenderness	: :Extremely: :tender	: :Very :tender	: :Tender	: :Plus: :	: :Medium: :	: :Minus: :	: :Fair: :	: :Tough :	: :Very :tough	: :Extremely: :tough	: :	: :

Table 9. Mean palatability scores¹ for 20 samples of top round cooked for 30 minutes.

Round number	Sample number	Aroma	Flavor	Juiciness	Tender-ness
I	2	8.2	6.5	4.0	7.7
	3	8.2	6.8	4.3	8.2
	Average	8.2	6.7	4.2	8.0
II	5	8.2	7.2	3.2	8.5
	8	7.8	7.0	4.0	7.7
	Average	8.0	7.1	3.6	8.1
III	10	8.4	6.8	3.0	7.8
	12	8.6	7.2	2.8	7.6
	Average	8.5	7.0	2.9	7.7
IV	14	8.3	7.6	3.0	6.8
	16	8.3	7.2	3.4	6.0
	Average	8.3	7.4	3.2	6.4
V	17	8.5	7.5	3.5	7.5
	20	8.3	7.5	3.8	7.5
	Average	8.4	7.5	3.7	7.5
VI	21	8.8	8.0	4.4	7.8
	24	8.2	6.8	3.4	6.8
	Average	8.5	7.4	3.9	7.3
VII	26	7.2	5.8	3.6	8.6
	27	7.8	7.0	3.8	6.6
	Average	7.5	6.4	3.7	7.6
VIII	30	8.0	6.8	3.5	7.0
	31	8.0	7.2	3.8	6.2
	Average	8.0	7.0	3.7	6.6
IX	33	8.2	7.6	5.2	7.2
	35	8.0	7.4	3.4	7.2
	Average	8.1	7.5	4.3	7.2
X	37	8.3	7.5	3.3	7.3
	39	8.2	7.7	3.2	6.7
	Average	8.3	7.6	3.3	7.0

¹ Maximum score possible, 10.

Table 10. Mean palatability scores¹ for 20 samples of top round cooked for 45 minutes.

Round number	Sample number	Aroma	Flavor	Juiciness	Tender-ness
I	1	8.3	6.8	3.5	8.8
	4	8.2	7.0	4.8	8.5
	Average	8.3	6.9	4.2	8.7
II	6	8.0	6.8	3.5	9.2
	7	8.0	7.2	3.0	8.7
	Average	8.0	7.0	3.3	9.0
III	9	8.4	7.8	2.8	9.0
	11	8.4	7.2	2.6	8.0
	Average	8.4	7.5	2.7	8.5
IV	13	8.3	7.5	3.0	8.3
	15	8.5	7.8	3.8	7.2
	Average	8.4	7.7	3.4	7.8
V	18	8.5	7.8	2.8	8.3
	19	8.3	7.0	2.5	8.0
	Average	8.4	7.4	2.7	8.2
VI	22	8.6	7.4	3.4	7.4
	23	8.2	6.6	2.8	8.4
	Average	8.4	7.0	3.1	7.9
VII	25	8.2	7.4	4.0	8.2
	28	8.0	7.4	4.2	7.4
	Average	8.1	7.4	4.1	7.8
VIII	29	8.3	7.5	2.8	9.0
	32	8.3	7.0	3.3	7.7
	Average	8.3	7.3	3.1	8.4
IX	34	8.2	7.6	3.8	7.2
	36	8.0	7.2	3.0	5.6
	Average	8.1	7.4	3.4	6.4
X	38	8.3	7.8	3.3	8.3
	40	8.3	7.5	2.8	7.5
	Average	8.3	7.7	3.1	7.9

¹ Maximum score possible, 10.

Table 11. Mean shear values for 40 samples of top round.

Round	Sample No.	Shear value			Round	Sample No.	Shear value		
		Raw	Cooked				Raw	Cooked	
			30'	45'				30'	45'
I	1	16.6	--	7.6	VI	21	20.1	11.9	--
	2	25.1	19.9	--		22	29.6	--	14.6
	3	32.1	21.3	--		23	27.0	--	17.3
	4	29.2	--	17.4		24	32.4	20.2	--
	Av.	25.8	20.6	12.5		Av.	27.3	16.1	16.0
II	5	19.6	14.1	--	VII	25	23.2	--	7.6
	6	15.6	--	8.7		26	26.9	18.8	--
	7	24.3	--	13.2		27	34.6	13.9	--
	8	17.9	18.7	--		28	25.1	--	16.2
	Av.	19.4	16.4	11.0		Av.	27.5	16.4	11.9
III	9	16.0	--	9.6	VIII	29	24.5	--	13.4
	10	23.5	17.7	--		30	24.0	17.7	--
	11	27.1	--	20.9		31	30.4	25.0	--
	12	24.9	20.5	--		32	19.4	--	21.6
	Av.	22.9	19.1	15.3		Av.	24.6	21.4	17.0
IV	13	15.0	--	7.7	IX	33	18.1	13.9	--
	14	18.5	9.6	--		34	30.3	--	19.1
	15	21.6	--	14.0		35	29.8	30.4	--
	16	26.7	12.9	--		36	19.5	--	25.2
	Av.	20.5	11.3	10.9		Av.	24.4	22.2	22.2
V	17	23.7	9.4	--	X	37	27.2	11.8	--
	18	23.7	--	13.0		38	30.7	--	19.7
	19	23.0	--	15.8		39	28.6	22.7	--
	20	39.4	13.7	--		40	18.3	--	15.0
	Av.	27.5	11.6	14.4		Av.	26.2	17.3	17.4

Table 12. Mean collagen nitrogen values determined by the enzyme method for raw and cooked samples from 10 top rounds.

Round No.	Sample No.	Cooked			Round No.	Sample No.	Cooked		
		Raw	30'	45'			Raw	30'	45'
I	1	7.8	--	6.1	VI	21	9.5	5.4	--
	2	7.2	7.2	--		22	9.0	--	6.2
	3	7.8	7.2	--		23	9.0	--	7.2
	4	7.6	--	7.2		24	9.2	7.5	--
	Av.	7.6	7.2	6.7		Av.	9.2	6.5	6.7
II	5	8.6	6.0	--	VII	25	8.2	--	6.6
	6	7.4	--	5.2		26	9.5	6.5	--
	7	9.0	--	6.5		27	10.1	8.0	--
	8	8.9	6.2	--		28	9.1	--	8.2
	Av.	8.5	6.1	5.9		Av.	9.2	7.3	7.4
III	9	9.5	--	5.8	VIII	29	10.6	--	9.9
	10	8.4	6.5	--		30	10.3	9.7	--
	11	9.5	--	6.8		31	10.6	10.1	--
	12	8.3	6.4	--		32	9.3	--	9.2
	Av.	8.9	6.5	6.3		Av.	10.2	9.9	9.6
IV	13	8.2	--	5.5	IX	33	11.1	10.5	--
	14	8.5	6.0	--		34	10.7	--	10.0
	15	8.8	--	7.5		35	11.2	9.5	--
	16	9.7	6.3	--		36	11.3	--	9.8
	Av.	8.8	6.2	6.5		Av.	11.1	10.0	9.9
V	17	10.6	6.4	--	X	37	11.5	10.2	--
	18	10.6	--	5.9		38	11.1	--	9.9
	19	12.0	--	7.0		39	12.2	10.3	--
	20	8.5	7.3	--		40	12.5	--	9.9
	Av.	10.4	6.9	6.5		Av.	11.8	10.3	9.9

Table 13. Mean collagen nitrogen values determined by the blender-centrifuge method for raw and cooked samples from 10 top rounds.

Round:Sample:		Cooked			Round:Sample:		Cooked		
No. :	No. :	Raw :	30' :	45' :	No. :	No. :	Raw :	30' :	45' :
I	1	9.4	--	9.4	VI	21	9.5	9.1	--
	2	8.9	9.5	--		22	8.9	--	9.5
	3	10.5	9.4	--		23	10.3	--	9.1
	4	10.6	--	9.0		24	10.6	9.5	--
	Av.	9.9	9.5	9.2		Av.	9.8	9.3	9.3
II	5	10.1	9.0	--	VII	25	11.0	--	9.0
	6	10.2	--	8.4		26	11.5	9.6	--
	7	10.2	--	8.5		27	11.1	9.9	--
	8	10.3	10.0	--		28	10.7	--	8.7
	Av.	10.2	9.5	8.5		Av.	11.1	9.8	8.9
III	9	11.2	--	8.8	VIII	29	11.1	--	9.2
	10	9.6	9.9	--		30	10.4	10.4	--
	11	10.9	--	8.3		31	11.0	9.7	--
	12	10.7	9.4	--		32	10.5	--	9.4
	Av.	10.6	9.7	8.6		Av.	10.8	10.1	9.3
IV	13	10.1	--	9.0	IX	33	10.7	9.7	--
	14	9.9	8.6	--		34	11.0	--	9.6
	15	9.8	--	8.2		35	11.1	9.5	--
	16	10.2	8.8	--		36	11.5	--	9.5
	Av.	10.0	8.7	8.6		Av.	11.1	9.6	9.6
V	17	11.5	8.9	--	X	37	10.2	10.6	--
	18	10.8	--	8.5		38	11.3	--	9.3
	19	11.2	--	8.8		39	9.8	9.1	--
	20	10.9	9.4	--		40	10.3	--	8.8
	Av.	11.1	9.2	8.7		Av.	10.4	9.9	9.1

Table 14. Mean total nitrogen values for raw and cooked samples from 10 top rounds.

Round:Sample:		Cooked			Round:Sample:		Cooked		
No. :	No. :	Raw :	30' :	45' :	No. :	No. :	Raw :	30' :	45' :
I	1	3.80	--	6.04	VI	21	3.87	6.06	--
	2	3.74	5.84	--		22	3.89	--	6.31
	3	3.64	6.26	--		23	4.03	--	6.48
	4	3.62	--	6.40		24	4.11	6.32	--
	Av.	3.70	6.05	6.22		Av.	3.98	6.19	6.40
II	5	3.59	6.43	--	VII	25	3.74	--	6.42
	6	3.67	--	6.26		26	3.81	6.46	--
	7	3.82	--	6.53		27	3.90	6.50	--
	8	3.82	6.30	--		28	4.05	--	6.55
	Av.	3.73	6.37	6.40		Av.	3.88	6.48	6.49
III	9	3.58	--	6.14	VIII	29	3.78	--	6.02
	10	3.88	6.14	--		30	3.81	5.89	--
	11	3.88	--	6.69		31	3.92	6.47	--
	12	3.84	6.52	--		32	4.00	--	6.58
	Av.	3.80	6.33	6.41		Av.	3.88	6.18	6.30
IV	13	3.63	--	5.95	IX	33	3.63	5.96	--
	14	3.83	6.44	--		34	3.82	--	6.25
	15	3.82	--	6.38		35	3.87	6.33	--
	16	3.96	6.28	--		36	3.80	--	6.40
	Av.	3.81	6.36	6.17		Av.	3.78	6.15	6.33
V	17	3.64	6.16	--	X	37	3.70	5.88	--
	18	3.87	--	6.50		38	3.67	--	6.52
	19	3.87	--	6.53		39	3.72	6.33	--
	20	3.99	6.39	--		40	3.60	--	6.40
	Av.	3.84	6.28	6.52		Av.	3.67	6.11	6.46

COMPARISON OF PALATABILITY SCORES AND SHEAR VALUES
WITH COLLAGEN NITROGEN VALUES FOR BEEF

by

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Tenderness of meat is one of the most desired qualities. Lean meat consists essentially of muscle fiber and a variable amount of connective tissue, both having some role in the tenderness of meat. Present methods for the chemical analysis of collagen, the major constituent of connective tissue, give inconsistent results and are time consuming. It was deemed worthwhile to study the effect on collagen nitrogen values by modifying a blender-centrifuge method that has been used in this laboratory for determining the collagen content of meat. This was done by the use of a phosphate wash and the addition of a proteolytic enzyme in the steps required for separation of soluble proteins from the collagenous material. The collagen nitrogen values determined by the modified or enzyme method were compared with those determined by the blender-centrifuge method. Relationships of collagen nitrogen values to tenderness scores and shear values also were studied.

Forty paired steaks from ten top rounds, graded U. S. Good, were cooked in a pressure cooker (10 p.s.i.g.) for 30 and 45 minutes. They were compared for aroma, flavor, juiciness, tenderness, shear force and collagen nitrogen content. Aroma, flavor, juiciness and tenderness were rated on a 10 to one scale by a palatability panel of six members. Collagen nitrogen values were determined chemically by the blender-centrifuge and the enzyme methods. Correlation coefficients were used to study relationships between collagen nitrogen values, shear values and tenderness scores. The t-test was applied to data to study differences between collagen nitrogen values obtained by the two methods of

determination.

There were no noticeable differences in aroma, flavor and juiciness scores which could be attributed to the cooking times used in this study. However, the steaks that were cooked 30 minutes were rated slightly less tender by the panel than those that were cooked 45 minutes.

For all rounds the average shear value for the cooked meat was less than that for the raw meat. There was no relationship between shear values for raw meat and meat cooked 30 minutes, but there was a slight relationship between shear values for raw meat and those for meat cooked 45 minutes.

Similar to the shear values for raw meat and that cooked for 30 minutes, there was no relationship between shear values and tenderness scores for the meat cooked 30 minutes. On the other hand, tenderness scores were definitely related to shear values for meat cooked 45 minutes.

A near significant correlation coefficient was obtained for shear values and collagen nitrogen values determined by the enzyme method for meat cooked 30 minutes, and a highly significant coefficient when the meat was cooked 45 minutes. However, there was no relationship between shear values and collagen nitrogen values for meat cooked for either 30 or 45 minutes when the collagen nitrogen values were determined by the blender-centrifuge method. Moreover, there was no relationship between shear values and collagen nitrogen values for raw meat determined by either method.

Tenderness scores and collagen nitrogen values determined by the enzyme method were significantly related for samples cooked

both 30 and 45 minutes. There was no relationship when the same factors were compared using the blender-centrifuge method for determination of collagen nitrogen.

When the data for all samples were pooled for the t-test, collagen nitrogen values were significantly ($P < .001$) higher for both raw and cooked beef when determined by the blender-centrifuge method than when determined by the enzyme method. A change was made in the enzyme method after the first 24 samples were run (chloroform was added before incubation); thus, these data were analyzed separately from the last 16 samples. Under these circumstances, the t-test indicated that the collagen nitrogen values for the first 24 samples were significantly ($P < .001$) higher when measured by the blender-centrifuge method than when determined by the enzyme method, but for the last 16 samples there were no significant differences between the collagen nitrogen values obtained by the two methods.

In this experiment, there was higher correlation between shear values for raw and cooked meat, shear values and tenderness scores and shear values and collagen nitrogen values (enzyme method) when these factors were compared for meat that had been cooked for 45 minutes than for that cooked 30 minutes. Also, better relationships between collagen nitrogen values and the factors listed above were found when the enzyme method rather than the blender-centrifuge method was used for the determination of collagen nitrogen.