RAPID DETERMINATION OF COLLAGEN BY CENTRIFUGATION TECHNIQUE

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TABLE OF CONTENTS

INTRODUCTION

The science of food research involves a serious problem in selecting suitable methods for determining such evasive characteristics of palatability as tenderness, aroma, and flavor. While every research laboratory is striving to standardize desirable methods for constructive criticism of food products, it is difficult to correlate the data of one laboratory with another and often impossible to reproduce it. In the study of the methods of measuring the tenderness of meat, both objective and subjective means have been tried. Four of the methods which have been used are: (1) rating by a palatability committee, (2) histological examination, (3) measuring the mechanical shear or penetration force, and (4) chemical determination of the collagen and elastin content.

Many laboratories have found a definite relation between several of the four methods in their studies of the tenderness of both raw and cooked meat. If, then, a method could be devised which would be short, reproducible, and reliable, the comparison of data from the various laboratories would be simplified.

Because of the apparent relationship which has been observed between the amount of collagen in beef tissue and the degree of tenderness, it was imperative that a rapid, reproducible analytical method be developed. Several chemical methods for the determination of collagen in beef tissue have been in use. All involve the conversion of collagen to gelatin and the subsequent

determination of its nitrogen expressed as percent of total nitrogen. The methods, however, were long and tedious and not adapted to laboratory routine where many samples are analyzed. It was the purpose, then of this study to devise a chemical method for the determination of collagen which would fit the qualifications for laboratory use, that is, to be reliable and capable of being reproduced and to require a minimum amount of time.

REVIEW OF LITERATURE

Many laboratories have used the subjective method of determining tenderness in meat. Cline, Cover, and Whipple (1930) gave an extensive report on palatability scores for meats, using specially planned score cards. Cover (1936) introduced a paired-eating method devised to determine differences in tenderness between paired samples. It is especially valuable in comparing methods of cooking. In 1940 the method was modified somewhat to increase its usefulness. Individual preference for defining tenderness in meat is an important factor in meat research, but it is often criticized because of individual whims and prejudices which make it difficult to reproduce the data.

Paul, Lowe, and McClurg (1944) used the method of histological examination in combination with the palatability tests to ascertain the effect of ripening of beef. A noticeable change in the collagenous connective tissue was found by Ramsbottom, Strandine, and Koonz (1945) when the cooked samples were observed by histological examination of meat. Through histological examination, changes of structure which accompany the tenderizing

effect in meat may be observed.

According to Mackintosh, Hall, and Vail (1936), tenderness of meat was probably first studied by Lehmann and co-workers at the Hygiene Institute of Wurzburg where a mechanical means for measuring the tenderness of meat was developed. These workers believed that tenderness was correlated with the amount of connective tissue in the flesh. A chemical method for separating the proteins of the connective tissue from the remaining proteins had been devised earlier.

In 1928, Warner reported on the "mouse trap", a mechanical contrivance for the measurement of tenderness of meat developed at Beltsville. Tressler, Birdseye, and Murray (1932) described two methods for determining tenderness mechanically: (1) a cutting gage or puncturing apparatus, and (2) a penetrometer. Both were used in their study on the relative tenderness of chilled and quick frozen beef. They found the two methods sufficiently sensitive to indicate difference of tenderness in different cuts of meat from the same carcass. Noble, Halliday, and Klaas (1934) used a penetrometer to study the tenderness and juiciness of cooked meat. Mackintosh, Hall, and Vail (1936) described the use of the "Warner-Bratzler meat tenderness shear" in their work and later in 1944 Hall et al. reported a high correlation between the mechanical shear, palatability, and chemical methods for determining tenderness. Satorius and Child (1938) used a shearing device modified by Child to compare tenderness of beef and pork muscles cooked at varying oven temperatures. Satorius and Child

(1938a) also used this apparatus to detect changes in tenderness of beef cooked to various internal temperatures. They found a high correlation between subjective methods of determining tenderness and the shear data. Winkler (1938) developed a mechanical method for determining tenderness in a study concerning the relation between pH and tenderness of beef.

Mitchell, Zimmerman, and Hamilton (1926-1927) refined the early method for determining collagen and elastin, the proteins of the connective tissue. Mitchell, Hamilton, and Haines (1928) further modified the method of extracting collagen and applied the method to cooked meat but did not find it successful. Bell, Morgan, and Dorman (1941) further revised the method and found it successful when used in determining the collagenous content of raw as well as cooked beef. Hall et al. (1944) also modified the Mitchell, Hamilton, and Haines method and found it correlated with the shear and palatability determinations for tenderness.

Hall et al. (1944) observed that by determining the water insoluble fraction of raw rib eye, an approximation of the collagen content could be calculated, as collagen was nearly 21 percent of the fraction. The theory was presented then that the water-insoluble protein served as a structure of which collagen was the binding agent. To substantiate this theory, it was pointed out that during ripening, the collagen content decreased as more of the proteins became water soluble.

Chamical tests other than quantitative determination of collagen and elastin have been used. Satorius and Child (1938a) used in combination with the mechanical shear, a determination of

press fluid, total moisture and ether extract. They found no correlation between the press fluid and juiciness or aroma.

METHODS AND MATERIALS

The methods for the determination of collagen in beef tissue are based upon the fact that collagen is hydrolyzed to gelatin. Scotchard, Oncley, Williams, and Brown (1944) have made a study of the physical behaviors of solutions of degraded gelatin and concluded that collagen consists of long chains of polypeptide residues. Because the bonds between these residues hydrolyzed at different rates, gelatin was formed when the more reactive bonds were hydrolyzed. Real gelatin consists of mixtures of molecules which are the length of a chain between two reactive bonds and may include every possible peptide from the single amino acids to chains containing one less residue than the parent molecule. To determine the amount of connective tissue present, a separation must be made between the elastin and the collagen, and these two from the other meat proteins. The elastin separation depends on trypsin digestion from the residue from which the collagen has been extracted. The separation and determination of elastin were not considered in this study.

Lean meat is essentially fiber held together by connective tissue (elastin and collagen). Elastin and collagen possess individual chemical characteristics and may be separated from each other because of these special properties. Because this connective tissue appears to cause meat to be less tender, a measure of the amount of connective tissue present should indicate a

negative correlation with tenderness. Upon cooking, part of the collagen may be hydrolyzed and the tenderness thus increased. However, if a cut of meat contains very little collagen, the cooking process may not make the piece more tender but will probably decrease the tenderness due to coagulation of muscle protein, according to Satorius and Child (1938a).

The cuts selected for this series were those which contain a large quantity of the connective tissue such as pieces which might be used for "pot roasts". It is evident that the cuts of meat selected for the chemical determination of collagen in relation to measuring tenderness should be those which are comparatively high in collagen content.

The outside fat was removed from the selected cuts. They were then finely ground twice in a hand grinder, packed in square eight-ounce bottles, and quick frozen in the sharp freezer (-23°C.) until ready for use. The samples to be used were thawed overnight in the cooler, weighed, and analyzed.

Mitchell, Hamilton and Haines Method

The Mitchell, Hamilton and Haines (1928) water extraction method for collagen depends upon the separation of water-soluble proteins by means of a 100-mesh sieve. The 100-gram samples were placed in ball mills with 300 ml. of water and ground with two pounds of balls for 90 minutes, then washed on a 100-mesh sieve. Seven washings were recommended; each time the residue from the sieve was taken up in a beaker with 150-300 ml. of water (45-50°C.) and stirred with a glass rod until completely dis-

persed. All the filtrates were rejected. Following the seventh washing, the residue was dispersed in a 800 ml. beaker with 400 ml. of water and autoclaved for two hours at 15 pounds pressure. At the conclusion of the autoclaving process, the pressure of the autoclave was allowed to reduce slowly over a 30-minute period. The supernatant liquid was decanted through a linen filter and the filtrate collected. The residue was washed into a beaker with 100 ml. of boiling water and filtered. The washings were repeated five times or until a negative biuret test was obtained. The filtrate was made to a liter volume with distilled water and 100-ml. aliquots taken for nitrogen determination by the Kjeldahl method.

Bell, Morgan and Dorman Method

In attempting to apply the Mitchell, Hamilton and Haines method of collagen determination to cooked meat, Bell, Morgan and Dorman (1941) noticed a large amount of granular material which passed through the 100-mesh sieve each time the tissue from the ball mill was washed. When this granular material was recovered from the filtrate, washed free of the water-soluble protein, and recombined with the residue on the sieve, a considerable increase in collagen nitrogen was noted. These observations were confirmed in this laboratory.

Accordingly, the filtrate from the sieving process was filtered through a Buchner funnel with a linen filter cloth. Following the autoclaving of the combined residues from the sieve and the Buchner funnel, the liquid was tested for tyrosine and

tryptophane. Negative results showed that no proteins other than gelatin were present in the filtrate. The Bell, Morgan and Borman method gave more consistent results for collagen determinations in the same cuts than the Mitchell, Hamilton and Haines method which discarded the granular residue.

Tyrosine and tryptophane tests made upon the washings of the autoclaved residue led to the conclusion that water-soluble proteins were less easily washed from cooked than from raw samples. The filtrate from the autoclaved residue was concentrated to 500 ml. It often appeared cloudy and was cleared by the addition of 10 ml. of 0.1 N H_2SO_4, refluxing on a steam bath for two or more hours, and filtering before the aliquot was taken.

The cooked meat was found to contain less collagen than the samples of raw meat. The collagen apparently was lost, due to hydrolysis during cooking, and the hydrolysis appeared to be greater during the longer cooking periods. It was concluded that the method as described could be used as a measure of change in tenderness produced by cooking.

The increase in collagen nitrogen yield and the ability to check results with the improved method of Bell, Morgan and Dorman made the chemical determination more useful. However, the time involved in a single pair of samples still made the method unwieldly and time consuming for control measures involving large numbers of samples. A more rapid method is necessary in following the change in structure and tenderness of meat during cooking, storage, freezing, or aging on an extensive scale.

Centrifuge Method

Observations made by Hall et al. (1944) concerning water imbibition and isoelectric points showed that when meat in a suspension of water is brought to its isoelectric point, the nonsoluble proteins may be separated from the liquid by centrifuging. This fact suggested a new method of separation for collagen from the other meat proteins without involving the use of the sieve. Using this fact then as a basis, the new method was developed.

Duplicate 10-gram-samples of ground beef tissue were homogenized in a Waring blendor with 40 ml. of water for four minutes. In order to keep the temperature low, the water and blendor apparatus were put into the cooler before this operation. After four minutes, the blendor was stopped and the previously determined amount of normal sulfuric acid was added to adjust the meat to its isoelectric point (pH 5); the mixture then was blended for an additional one minute.

Adjustment of pH

In order to determine the amount of normal sulfuric acid necessary to adjust the meat suspension to pH 5, a separate assay for that purpose was run. Five grams of tissue and 20 ml. of water were mixed in a four-ounce square bottle with a small motor-driven stirrer. The pH of this suspension was determined with a glass electrode assembly which could be inserted into the bottle. A tentative amount (0.15-0.2 ml.) of the acid was added and mixed

with the suspension. The pH was again determined, and by proportion the theoretical amount of acid was calculated and added to a fresh portion of meat and mixed. The pH was again determined and further adjustments made it necessary to bring the pH to five. Proportional amounts were added when larger samples were used.

Centrifuge tubes, 40 ml. capacity, were used to centrifuge the homogenate. The speed of the centrifuge was on the average 3,000 r.p.m., and each time the centrifuge was allowed to run five minutes and stop without the use of a brake.

Five washings were made on the raw sample and six on the cooked sample. After the initial liquid had been centrifuged and drained, the tube was refilled with water (45-50°C.) and the residue thoroughly mixed with a stirring rod, making sure all the fibers were well dispersed. A biuret test (Hawk, Oser and Summerson, 1947) was made upon the final washing to insure the removal of all water-soluble proteins from the residue. The fifth washing was negative for the raw sample. A sixth washing was found to be necessary for the cooked sample.

The residue was dispersed with 80 ml. of water in a 125 ml. Erlenmeyer flask and stoppered with a cotton plug. The flask and contents were then autoclaved two hours at 15 pounds pressure and allowed to reduce pressure gradually. The autoclaved sample was decanted into centrifuge tubes, centrifuged, and the supernatant was poured into a Kjeldahl flask. The remaining contents of the flask were transferred to the centrifuge tubes and washed with boiling water five times by centrifugation. Each time the tubes

were immersed in boiling water two minutes and the contents stirred. The tubes were centrifuged about three minutes. The supernatants in each case were combined in the Kjeldahl flask.

With the raw samples, the residue packed in the bottom of the tubes so firmly that the liquid could be poured from the residue; however, with the cooked samples a filter had to be used to prevent residue from being carried with the filtrate. Before the supernatant liquid was poured from the residue, it was examined closely and a filter used if it appeared necessary.

Tryptophane tests were made on several autoclaved samples in order to be assured of the complete removal of non-collagen proteins. Under the prescribed procedure no measurable amount of tryptophane was found to be present.

The residue was discarded and the entire filtrate used for collagen nitrogen determination by the Kjeldahl method. Collagen nitrogen was expressed as percent of total nitrogen.

Tryptophane tests were made upon the autoclaved samples to determine whether the nitrogen present was of gelatin origin. Gelatin contains a very small amount of tyrosine and no tryptophane. It was concluded if a positive test for tryptophane was obtained, proteins other than gelatin would be present. May and Rose (1922) determined the tryptophane content of several proteins. They used the p-dimethylamino benzaldehyde reagent which produced a blue color when added to a mixture of proteins. Folin and Marenzi (1929) determined the tyrosine and tryptophane content of proteins, using a micro method which was a modifi-

cation of Folin and Ciocalteu (1927). Bell, Morgan and Dorman (1941) found that the method of May and Rose gave a slightly higher tryptophane content than that of Folin and Marenzi, but they concluded that it was not necessary to determine the amount of tryptophane and tyrosine present as a part of their modified method.

When Holm and Greenbank (1923) modified the May and Rose tryptophane determination to allow eight to ten days for maximum color development, they obtained more consistent results. They also found that casein was a better standard in the determination than pure tryptophane. However, even with the use of casein, there was some indication of loss of reactive tryptophane with the development of a reddish color rather than the blue (Sullivan, Milone and Everitt, 1938).

The tryptophane calculation was based upon the fact that casein contains a measurable amount of tryptophane which varies with different preparations.

EXPERIMENTAL RESULTS

A sample of round was selected to be used in the determination of collagen in raw beef by the three methods previously described. Comparison of the data indicated that the Bell, Morgan and Dorman and the centrifuge method both gave results higher than that obtained by the Mitchell, Hamilton and Haines method (Sample 4 in Table 1). However, the results by the Mitchell, Hamilton and Haines method did not deviate so widely from the

Table 1. Adjustment of pH for centrifuge method for collagen nitrogen and comparison with results obtained by other methods.

Sample	pH	Normal[a] H_2SO_4 ml.	Collagen nitrogen in percent of total nitrogen		
			Centrifuge	Bell	Mitchell
		Raw Sample			
1	5.46	0.20	11.7	11.4	9.8
2	5.58	0.32	13.4	13.4	---
3	6.35	0.72	9.1	11.6[b]	---
4	5.68	0.28	13.9	10.6	---
		Cooked Sample			
4	5.92	0.56	12.2	9.23	---
5	5.78	0.36	11.2	9.65	---
6	5.74	0.28	10.8	8.10	---

[a]Volume required to adjust sample to isoelectric point.

[b]After adjustment to pH 5.

other methods as was reported by Bell, Morgan and Dorman (1941), who found differences between the two methods amounting to 50 percent. Closer agreement between the results obtained by the two methods in this laboratory indicated that the Mitchell, Hamilton and Haines method is subject to considerable deviation in results due to the variation of individual technique in different laboratories.

In order to test the reproducibility of the centrifuge method, a series of samples of the same round were analyzed. The collagen content for the series is shown in Table 2.

Table 2. Reproducibility of the results obtained by the centrifuge method.

Series No.	Collagen nitrogen in percent of total nitrogen
1	13.1
2	11.9
3	11.9
4	13.1
5	11.9
Average	12.4
Average deviation from mean	0.6

It was concluded that reproducible results could be obtained by the centrifuge method to determine the collagen nitrogen of raw beef.

Previous experience in this laboratory has indicated that the Mitchell, Hamilton and Haines method did not give satisfactory duplications. For example in a series of 11 samples, difference in duplication ranged from zero to 1.5 percent collagen nitrogen, with an average of 0.6 whereas, differences in duplication by the Bell, Morgan and Dorman method on raw samples averaged 0.4 percent, and on cooked samples 0.2 percent. For the centrifuge method, the differences averaged 0.3 on raw samples and 0.8 percent on cooked samples.

Results by the centrifuge method tended to run somewhat higher than those obtained by the Bell, Morgan and Dorman method. This difference may be explained by the fact that more effective separation of water-soluble protein was obtained by the centrifuge method. Although the Bell, Morgan and Dorman method re-

covered much of the residue passed through the sieve by the Mitchell, Hamilton and Haines method, it was not all retained by a linen filter.

Dark-cutting Beef

Because of the physical differences between dark-cutting beef and normal beef, a dark sample was included in an experiment set up to determine the collagen content by the Bell, Morgan and Dorman method and by the centrifuge method. Hall et al. (1944) gave a description of dark-cutting beef and its characteristic physical properties as well as some chemical properties, especially its high pH. The lean might appear in color from brownish-red to purplish-black, with no abnormal appearance of the fat tissue but the texture of the beef was always sticky and gummy. This resulted in difficulty when an attempt was made to separate the water soluble proteins from the collagenous tissue. The normal procedure was followed for the Bell, Morgan and Dorman method in which 100-gram portions of meat were ball milled with 300 ml. of water. Eighteen balls were used as directed and the milling process was continued 90 minutes. When the samples were washed onto the sieve, the fibers were matted together with a gelatin like material which could not be separated. Washings were attempted, but when a sixth washing was completed, a reddish tinge persisted in the tissue, and fibers were still bound together by the gelatinous mass. An attempt was made to filter the colloidal suspension which passed through the sieve and retain the residue

as directed by the Bell, Morgan and Dorman method. The suspension was much too colloidal and passed through the linen filter, making it impossible to separate the granular residue. It was evident that the method was entirely unsatisfactory for the collagen determination of dark-cutting beef.

Therefore, the Bell, Morgan and Dorman method was modified to the extent of adjusting the tissue to pH 5 before the ball-milling process. Filtering and washing processes then yielded clear filtrates and the analysis was completed without difficulty. Results are indicated under Sample 3 in Table 1.

When the dark-cutting beef was extracted by the centrifuge method, no difficulty in separation occurred. The residue packed firmly in the bottom of the centrifuge tube and the filtrate was clear and drained easily each time a washing was made. The residue appeared white with no trace of a reddish tinge. The autoclaving and washings were conducted as described.

A comparison of the data obtained revealed that in this case alone, the ball-milling process gave a somewhat higher collagen nitrogen, 11.6 percent, than the centrifuge method which gave 9.2 percent of collagen for the same sample. It seems that this discrepancy might be explained by possible occlusion of water-soluble protein during flocculation caused by adjusting to the isoelectric point.

Cooked Beef

Having found the centrifuge method successful for the determination of collagen nitrogen in raw meat, the procedure was further extended to the analysis of cooked meat. In the process of meat cookery, three things should be accomplished in order for it to be successful: the flavor should be retained, the color changed, and the meat should increase in tenderness. The Committee on Preparation Factors, National Cooperative Meat Investigations, listed these palatability factors as important and dependent on the method of cooking in order that the best results might be obtained. To increase the tenderness, the soluble protein must be coagulated and the hydrolysis of the collagen to gelatin accomplished. Conclusions drawn from the study by Bogue (1923) indicated the important factors in converting collagen to gelatin to be temperature, time the meat is held at that temperature, and acidity of the meat. Temperatures around boiling induce the most rapid change and at temperatures above boiling, in a pressure cooker for example, the rate is still greater. With temperatures of 70-85°C. the rate is very slow.

In general, the meats are cooked by (a) dry heat and (b) moist heat. The more tender cuts of meat such as roasts and tender steaks are cooked by dry heat; these are the cuts with low connective tissue. Methods of cooking with dry heat include roasting, broiling, pan broiling, and cooking in fat. Cooking by moist heat is recommended for those less tender cuts which con-

tain a considerable amount of connective tissue, such as the round, rump, and flank. Braising and cooking in water are methods suggested for these cuts of meat.

These less tender cuts are those with high connective tissue which may be hydrolyzed under the correct conditions. Flavor is sacrificed in braising which necessitates long cooking in moisture. A tightly covered cooking utensil is required. If liquid is added to it in very small amounts, the meat retains its rich, brown color and original flavor, which are destroyed if the juices are diluted. Slow cooking is also essential for juicy, tender products.

Many of the less tender cuts which are used for stews and soups are cooked in large quantities of water. In these instances the attractive appearance and flavor development are sacrificed. The object, however, is reversed from other meat cookery processes. The purpose here is to extract the flavor and gelatin from the meat and make a rich broth. Meat cooked below boiling is juicy and the muscle fiber more tender than when boiled. Less collagen is extracted during simmering than during boiling.

It is, therefore, evident that the method for collagen analysis should be successfully applicable to cooked meat in order to be useful to meat cookery research.

Because people are most interested in making the less tender cuts more tender by cooking and still retaining the original flavor and attractive color, the meat used was cooked by the braising method.

The isoelectric point for cooked meat was not the same as for raw. The proteins had undergone physical and chemical changes during the process of hydrolysis and heat coagulation which altered the isoelectric point as well as the pH.

Because the centrifuge process depended entirely upon adjusting the water suspension to the isoelectric point, a series of samples was set up to determine the isoelectric point of cooked meat. The samples were steaks cut from the left side of the round. The pH was varied in the series by adding increments of 0.05 ml. of normal sulfuric acid. The points fell within one-tenth unit apart and the isoelectric points varied within approximately 0.2 unit. When the pH range was sufficiently extended, two minima appeared in the water inbibition curve, one falling closely to the value for raw beef muscle 5.07 found by Hall et al. (1944). The second point in all cases was higher, ranging from 5.2 to 5.4. The pH of three cooked samples was 5.67, 5.74, and 5.92, considerably higher than the normal pH of raw beef. The sample having the highest pH had the lowest isoelectric point; therefore, it does not seem feasible to predict the isoelectric point from the pH of the cooked meat.

The isoelectric point ranged from pH 5.18 to 5.40. Therefore, it is advisable to adjust the pH of cooked meat to a point somewhat higher than the isoelectric point of raw beef. A pH of 5.2 may be selected as a trial value which should not be far from the true isoelectric point of both the altered and unaltered proteins. After centrifuging, if the supernatant is turbid, further adjustment of pH may be necessary.

A three-pound round steak cut from three-fourths to one inch thick was selected because it was a common less tender cut of meat. It was seared in an electric broiler for seven minutes on each side and then put into a casserole with a tight-fitting lid and cooked for one hour at 350°F. with 60 ml. of water. The casserole was of Fraunfilter China made in Ohio. It was 10 inches in diameter and three and one-half inches in depth. When the cooking period was completed, the steak was removed, cooled, and the fat, gristle and bone removed in preparation for grinding. It was finely ground twice in a hand grinder, mixed and put in square eight-ounce bottles for storage at -23°C., the same procedure as was used for the raw sample.

Duplicate 100-gram samples of the braised meat were taken and the procedure followed as outlined by Bell, Morgan and Dorman (1941) for cooked meat. The juices were not recombined with the ground-cooked meat and in the ball-milling process, water was adsorbed, making the suspension very thick. A great deal of the residue washed through the sieve. The filtrate was colloidal in nature and passed through the linen filter rapidly. It was a laborious operation to lay down a layer of residue on the filter so that a clear filtrate could be obtained. The process involved four hours of filtering. The autoclaved residue was, also, difficult to filter, often requiring more than a full day for filtering alone.

In all instances when the Bell, Morgan and Dorman method was used for cooked meat analysis, it was necessary to clear the autoclaved filtrate with H_2SO_4. It was found that the colloidal ma-

terial could be coagulated by adding H_2SO_4 before evaporation to volume, thus avoiding refluxing as recommended by Bell, Morgan and Dorman (1941). Several hours of time were saved by avoiding this refluxing. Following the evaporation, the precipitate was filtered out. The filtrate was made up to 500 ml. volume and a 100 ml. aliquot was taken for Kjeldahl nitrogen.

The centrifuge method of determining collagen nitrogen was applied to cooked beef. Duplicate 10-gram samples of the braised meat were homogenized, the pH adjusted, mixed, and then allowed to stand for a few minutes before being transferred to the centrifuge tubes. The residue was washed with water (45-50°C.) six times after the original supernatant had been removed. The residue was autoclaved for two hours at 15 pounds pressure and washed in the prescribed manner, with the exception of the use of a paper filter when the supernatant was poured into the Kjeldahl flask. This added precaution was necessary because it had been found that the autoclaved sample of cooked meat failed to separate as clearly as that of the raw.

The collagen nitrogen yield of the round by the Bell, Morgan and Dorman method gave a 10.94 percent decrease in collagen content following the braising. The yield for the same sample when analyzed by the centrifuge method was 11.6 percent less than the raw collagen yield. The data obtained by the two methods compared favorably and indicated the usefulness of the new method.

Bell, Morgan and Dorman (1941) reported considerably higher conversion of collagen to gelatin by cooking than the data indicated here by the same method or by the centrifuge method. The

explanation of the variation is probably due to the methods of cooking. The method of simmering or boiling in excess water, extracts more of the collagen than the method of braising in which a small amount of water was used.

SUMMARY OF THE CENTRIFUGE METHOD

The method for the determination of collagen involving the use of the centrifuge as finally developed is essentially as follows:

Preparation of Sample. Put the meat twice through a hand grinder after the external fat and visible cartilage have been removed, mix thoroughly. Weigh duplicate 10-gram portions of ground sample into small-size Waring blendor jars, add 40 ml. of cool water, and homogenize for four minutes. Add the normal H_2SO_4 necessary to bring the suspension to the desired isoelectric point and blend for one minute.

Water Extraction. Rinse the homogenate into an Erlenmeyer flask and allow it to stand for 10 minutes. Decant the liquid into a 40 ml. centrifuge tube, centrifuge five minutes, and discard the filtrate. Rinse the remaining suspension into the tube, disperse the residue, and centrifuge. Drain, stir thoroughly with approximately 25 ml. of water (45-50°C.), and centrifuge. Wash in this manner five times for raw meat and six times for cooked meat. In order to determine whether the water-soluble proteins have been removed from the collagenous fiber, apply the biuret test to the final washing.

Hydrolysis. Wash the residue into a 125 ml. Erlenmeyer flask and disperse well, making the total volume 80 ml. Stopper with a cotton plug and autoclave at 15 pounds pressure for two hours. Allow the pressure to decrease gradually and leave the flask in the autoclave five minutes after the door has been opened. Pour the hot supernatant liquid into 40 ml. centrifuge tubes and centrifuge for three minutes (3000 r.p.m.). Drain the clear liquid into Kjeldahl flasks. Rinse the remaining autoclaved material into the centrifuge tube, centrifuge, and drain into the flask. Mix the residue with approximately 25 ml. of boiling water and place in a hot water bath for two minutes, stirring the mixture with a glass rod. Centrifuge. Repeat the washing five times, pouring supernatants into the Kjeldahl flask each time. Use entire filtrate for the collagen nitrogen determination. Determine total nitrogen on approximately two-gram samples of the original sample. Express collagen nitrogen as percent of total nitrogen.

Note: When the cooked samples were centrifuged following the autoclaving, it was found necessary to drain the supernatant liquid through a paper filter into the Kjeldahl flask in order to obtain a clear filtrate.

CONCLUSION

A method using the centrifuge was developed for the separation of collagen from other meat proteins. This method was found to give reproducible results when applied to raw and cooked beef. The method was compared with the Mitchell, Hamilton and Haines method and a revised method by Bell, Morgan and Dorman. Data obtained in this laboratory showed that the Bell, Morgan and Dorman and centrifuge methods gave higher collagen nitrogen results than the Mitchell, Hamilton and Haines method, but the range of increase was not so great as reported by Bell, Morgan and Dorman (1941). Results by the centrifuge method tended to run somewhat higher than those obtained by the Bell, Morgan and Dorman method.

The chief advantage of the centrifuge method is its adaptability to handling a large number of samples and its great saving of time and tedious manual operation.

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