Table II. Recoveries of Streptomycin from Commercial Feed Supplements

Replication	Supplement C,[a] P.P.M.	Supplement D,[b] Mg./G.
1	41,025	563.8
2	43,025	492.8
3	43,225	606.4
4	40.650	528.8
5	42,025	506.6
6	45,575	500.9
7	42,275	512.2
8	43,125	512.9
9	43,750	504.5
10	42,925	521.3
Av.	42,760	525.0
Mean dev.	1,013	24.8
Stand. dev.	1,378	33.5
Coeff. of variation	3.2	6.4
Av. % recovery	103.5	109.4

[a] Tag guarantee: 18.75 grams per pound of streptomycin (equivalent to 41,300 p.p.m.) and 6.25 grams per pound of procaine penicillin.
[b] Tag guarantee: Each gram contains the equivalent of 480 mg. of streptomycin base.

covery of 98.3% and a coefficient of variation of 5.7%.

Table II presents results obtained from the two commercially available feed supplements. Supplement C yielded results having a 103.5% average recovery with a coefficient of variation of 3.2%. Supplement D showed results having a 109.4% recovery and a coefficient of variation of 6.4%.

The recoveries of all samples were essentially quantitative and possessed a good degree of reproducibility. It is important to note that a 1-μg. error in determining the aliquot concentration can become equivalent in the final calculation to hundreds of micrograms. The exact magnitude of such an error will be determined by aliquot size and concentration of premix.

Any materials possibly interfering are eliminated by means of the blank. The concentration of streptomycin is directly proportional to the difference in absorbance obtained from a heated portion of alkaline aliquot and unheated acid aliquot. The difference in absorbance readings is attributed to the conversion of the streptose moiety of the streptomycin molecule to maltol.

The analyst has a fair amount of latitude in the use of this method. Modification as to the volume of extracting acid and the aliquot size is possible for the solution of specific problems with little or no loss in accuracy and reproducibility.

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Received for review April 10, 1961. Accepted May 29, 1961. Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University, the State University of New Jersey, Department of Agricultural Chemistry, New Brunswick, N. J.

MEAT TENDERNESS FACTORS

Determination of Collagen in Raw and Cooked Beef from Two Muscles by Alkali-Insoluble, Autoclave-Soluble Nitrogen and by Hydroxyproline Content

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Residual collagen, important in tenderness of meat, was measured in steaks of *L. dorsi* and *B. femoris* by micro-Kjeldahl nitrogen and by hydroxyproline following exhaustive extraction with alkali and conversion to gelatin by autoclaving. Collagen nitrogen values measured by hydroxyproline were consistently lower than those measured by micro-Kjeldahl in raw steaks, as well as in rare (61° C.) and well done (100° C.) steaks. Losses of collagen nitrogen were about the same in cooked steaks of the two muscles by each method of determination, but the two methods of assessment did not correspond. Losses were much greater in well done steaks than in rare steaks. *B. femoris* contained considerably more hydroxyproline and collagen than *L. dorsi*.

One of the factors believed to be involved in the tenderness or toughness of a piece of meat is the amount of residual connective tissue with collagen representing the major portion of this moiety. Studies on collagen from different species by Wohlisch (*16*), Gustavson (*6*), and Keech (*8*) demonstrated the effect of heat on this protein. When meat is cooked or heated, a fraction of the collagen is converted into gelatin; the amount not converted is believed to be one important factor in the over-all toughness of meat. Previous studies by Cover and Smith (*4*), Griswold and Leffler (*5*), and Irvin and Cover (*7*) indicate that the amount of residual connective tissue or collagen is dependent upon the method of cooking and the particular muscle or cut of meat. Irvin and Cover (*7*) found that *B. femoris* contained considerably more collagen than *L. dorsi*, and that the loss of collagen ranged from 20 to 25% when steaks from either muscle were cooked to an internal temperature of 61° C.

Numerous methods have been devised for the assessment of collagen. The method of Lowry, Gilligan, and Katersky (*9*) or some modification of this procedure is employed most frequently. This method depends upon the insolubility of collagen in dilute alkali and its conversion to gelatin on autoclaving. Collagen was found by weight in the original procedure, but Wilson, Bray, and Phillips (*15*) determined nitrogen in the autoclave-soluble fraction as a measure of collagen. Irvin and Cover (*7*) modified the method by using a more exhaustive extraction with water and alkali and then determining collagen by nitrogen in the autoclave-soluble fraction. This modification has given satisfactory data as far as consistency of duplicate samples is concerned, for cooked samples, as well as raw meat. When meat is heated, muscle proteins become more insoluble

Table I. Collagen Nitrogen Determined by Nitrogen and by Hydroxyproline Content in Steers and Bulls

Method of Determination and Meat Source	No. of Animals	Grams Collagen Nitrogen/100 Grams Total Nitrogen		
		Raw	61° C.	100° C.+
Longissimus dorsi				
Micro-Kjeldahl nitrogen				
Steers	10	1.69 ± 0.07[a]	1.18 ± 0.07	0.08 ± 0.02
Bulls	4	1.75 ± 0.25	1.05 ± 0.01	0.06 ± 0.01
All	14	1.70 ± 0.08	1.14 ± 0.06	0.07 ± 0.01
Hydroxyproline				
Steers	10	1.32 ± 0.13	0.76 ± 0.12	...
Bulls	4	1.40 ± 0.23	0.74 ± 0.01	...
All	14	1.34 ± 0.11	0.75 ± 0.09	...
Biceps femoris				
Micro-Kjeldahl nitrogen				
Steers	10	2.89 ± 0.11	1.86 ± 0.11	0.12 ± 0.02
Bulls	4	2.76 ± 0.15	1.82 ± 0.16	0.15 ± 0.05
All	14	2.86 ± 0.16	1.85 ± 0.09	0.13 ± 0.02
Hydroxyproline				
Steers	10	2.35 ± 0.21	1.32 ± 0.17	...
Bulls	4	2.32 ± 0.20	1.56 ± 0.18	...
All	14	2.34 ± 0.15	1.39 ± 0.13	...

[a] Mean and standard error.

Table II. Hydroxyproline Content of Raw Meat from Two Muscles of Steers and Bulls

Meat Source	No. of Animals	Grams Hydroxyproline/100 Grams Total Nitrogen	
		L. dorsi	B. femoris
Steers	10	1.59 ± 0.10[a]	2.29 ± 0.13
Bulls	4	1.60 ± 0.18	2.30 ± 0.17
All	14	1.59 ± 0.08	2.29 ± 0.10

[a] Mean and standard error.

Table III. Loss of Collagen Nitrogen during Cooking[a]

Method of Determination	Loss on Cooking from Raw Samples, %			
	L. dorsi		B. femoris	
	61° C.	100° C.+	61° C.	100° C.+
Micro-Kjeldahl nitrogen	32.9 ± 2.6[b]	95.9 ± 0.8	35.3 ± 2.3	95.5 ± 0.8
Hydroxyproline	44.0 ± 4.3	...	40.6 ± 5.0	...

[a] Average of 14 animals.
[b] Mean and standard error.

and thus it becomes more difficult to extract all of the noncollagen nitrogen in cooked samples. The difficulty of extraction increases as the time and temperature of cooking increase. Miller and Kastelic (*13*) pointed out the results of failure to extract all of the noncollagen nitrogen; they found that collagen determined by hydroxyproline corresponded closely to the total autoclave-soluble nitrogen following alkaline extraction of raw samples. Loyd and Hiner (*10*) studied the relation of hydroxyproline in alkali-insoluble protein and tenderness in raw samples of several beef muscles.

In the present study, collagen was determined by nitrogen and by hydroxyproline in the alkali-insoluble, autoclave-soluble fraction. The data seem especially pertinent because collagen was determined not only in raw meat, but in samples which had been cooked to an internal temperature of 61° C. (rare) and 100° C. (very well done).

Experimental Procedure

Meat used in this study was obtained from 10 steers and four bulls of about 16 months of age produced at Texas Agricultural Experiment Station No. 23. *Longissimus dorsi* and *Biceps femoris* muscles were removed from carcasses which had been aged for 7 days at 36° F. Steaks 1 inch thick were cut from each muscle, frozen at −20° F., and stored at 0° F.

Collagen was determined on raw steaks, as well as on comparable ones cooked by dry heat to an internal temperature of 61° C. and on others cooked by moist heat to 100° C. and held at this temperature for 25 minutes. The procedures for cooking are reported in detail by Cover and Hostetler (*3*).

Outside edges were trimmed from steaks prior to cooking. The cooked steaks were cut into convenient pieces and finely ground in a Hobart electric grinder. Raw steaks were trimmed, cut, and ground while frozen. All samples were placed in aluminum pans and frozen. Moisture was removed in a freeze-dryer and dry samples were ground to a fine powder with a mortar and pestle. Removal of the major amount of moisture and subsequent grinding permitted thorough mixing and resulted in a homogeneous sample. Preliminary studies indicated that samples of approximately 1.2 grams were appropriate for the raw and 61° C. steaks, but that 0.6 gram was better for the 100° C. samples. Optimum extraction of nitrogen was the criterion used in this case.

The samples were extracted according to the modified Lowry, Gilligan, and Katersky (*9*) procedure as reported by Irvin and Cover (*7*) with one alteration. Irvin and Cover (*7*) had samples in contact with alkali for about 26 hours; in the present work, samples were in dilute alkali for 40 hours. After autoclaving, the filtrates were cooled and made to a convenient volume. Nitrogen was determined on an aliquot of the filtrates by the Association of Official Agricultural Chemists (AOAC) micro-Kjeldahl method. Total nitrogen was determined on dried samples by the same method.

The hydroxyproline content was determined on hydrolyzates of the autoclave-soluble filtrates by the method of Neuman and Logan (*14*) as modified by Martin and Axelrod (*11*). An aliquot of each filtrate was pipetted into a glass ampoule and the contents were made 6*N* with respect to acid by adding hydrochloric acid. The ampoules were sealed in a flame and autoclaved at 15 pounds' pressure (120° C.) for 16 hours. Preliminary work had revealed that the amount of hydroxyproline released during hydrolysis reached a plateau after 15 hours of hydrolysis under these conditions, and that the amount of hydroxyproline remained constant in samples hydrolyzed as long as 70 hours. Hydrolysis of pure hydroxyproline, alone and in combination with meat, revealed no destruction of the amino acid under these circumstances.

Results and Discussion

The average collagen nitrogen values as determined for the 10 steers and four bulls by both nitrogen and hydroxyproline contents are given in Table I. It was necessary to convert hydroxyproline, which was assayed in micrograms of amino acid, to collagen nitrogen. Numerous workers, including Bergmann (*1*), McFarlane and Guest (*12*), and Chibnall (*2*), have reported that collagen or gelatin contains 13% of hydroxyproline. In addition, they had found the quantity of nitrogen in collagen or gelatin to range from 17.5 to 18.7%. The figure of 18.6% has been used by Griswold and Leffler (*5*) and previously in this laboratory by Cover and Smith (*4*). Thus, collagen was calculated from hydroxyproline content using 13% as the conversion figure. Collagen nitrogen was determined by taking 18.6% of the calculated collagen.

Raw steaks from *B. femoris* muscle

contained considerably more collagen than ones from *L. dorsi* (Table I). Raw steaks from *B. femoris* and *L. dorsi* were found to have 2.86 and 1.70 grams of collagen nitrogen per 100 grams of total nitrogen, respectively, by the micro-Kjeldahl method. These values agree fairly well with previous work. Cover and Smith (*4*) found the two muscles to have 3.16 and 2.01 grams of collagen nitrogen per 100 grams of total nitrogen. Irvin and Cover (*7*) reported *B. femoris* and *L. dorsi* to have values of 3.08 and 1.87 grams, respectively. The slightly lower amounts of the present study can probably be attributed to variation between animals as earlier investigations by Cover and Smith (*4*) and by Wilson, Bray, and Phillips (*15*) have indicated or to the solubilization of collagen during extended extraction with dilute alkali.

Collagen data for steers and bulls are reported separately in Table I to show that there were no differences between them. The hydroxyproline contents of the two muscles (Table II) are the same for steers and bulls. There seems to be very little difference between these steers and bulls, but generalizations from the small number of animals involved in this study are rather uncertain.

The study of Irvin and Cover (*7*) showed the loss of collagen on cooking to 61° C. Data in the present study (Table I) indicate that losses of collagen occurred when steaks were cooked to this same internal temperature, and that additional losses were sustained at an internal temperature of 100° C. The losses during cooking are expressed in percentage in Table III. Collagen nitrogen losses for the two muscles agree closely within the micro-Kjeldahl method and within the corresponding hydroxyproline data. There is considerable variation in amount of collagen converted to gelatin between animals when steaks are cooked to 61° C. Collagen nitrogen loss approaches 100% when steaks of either muscle are cooked to 100° C. and held there for 25 minutes, by either method of analysis.

When the two methods were compared, collagen nitrogen calculated from the hydroxyproline content of the alkali-insoluble, autoclave-soluble fraction was somewhat lower than that measured by micro-Kjeldahl nitrogen (Table I). In raw meat, the hydroxyproline method gave 82 and 79% of the values by the nitrogen method for *B. femoris* and *L. dorsi*, respectively. In steaks cooked to 61° C., the percentages were 75 and 66 for the two muscles, respectively. Data (Table I) show very small amounts of collagen nitrogen for steaks of either muscle cooked to 100° C. There are no values by the hydroxyproline method of assessment, because hydroxyproline was found in only two or three instances in the filtrates of these samples, and the amount was very small in these cases. Perhaps hydroxyproline could be measured if several samples of the same steak were combined, but this was not undertaken because of the time element. The lower amount of collagen nitrogen by hydroxyproline could be the result of selection of improper factors for conversion of hydroxyproline to collagen nitrogen.

However, it seems more likely that the disagreement lies in the extraction procedure. Any noncollagen nitrogen which is not soluble in water or dilute alkali and which becomes soluble on autoclaving would result in nitrogen figures representing materials other than collagen. On the other hand, extensive extraction with alkali may result in solubilization of some collagen. This might explain differences between the two methods.

Total hydroxyproline was determined on all raw samples (Table III). *B. femoris* contained more hydroxyproline than the *L. dorsi* steaks. In an effort to check on the accuracy of the method, all of the extracts and washings from duplicate samples of a raw *B. femoris* muscle were analyzed for hydroxyproline. The water extracts contained 2.9%, the alkali extracts had 9.9%, and the autoclave-soluble filtrate contained 85.4% of the total hydroxyproline. This means that 98.2% of the total could be accounted for in the various soluble fractions. The residue was not analyzed.

Further efforts may result in more accurate methods of estimating collagen, but at the present time hydroxyproline seems to be a more reliable indicator of this protein in raw and cooked meat than nitrogen determination of the alkali-insoluble, autoclave-soluble fraction. This seems especially pertinent in cooked samples because of the decreased solubility of muscle protein, or noncollagen nitrogen, in these samples.

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Received for review March 13, 1961. Accepted May 12, 1961. Study was supported in part by the Animal Husbandry Research Division, Agricultural Research Service, U. S. Department of Agriculture.