

It is thus obtained in compact cubes, m. p. 233° - 234° (corr.), slightly soluble in cold acetone, warm benzene or amyl acetate, easily soluble in warm acetone or boiling nitro-benzene.

Calculated for $C_{15}H_{11}O_3N_3$: C, 64; H, 3.9; N, 14.9. Found: C, 64, 64.1; H, 3.9, 4; N, 14.7.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE
UNIVERSITY OF ILLINOIS.]

THE CHEMISTRY OF FLESH.

(SECOND PAPER.)¹

IMPROVED METHODS FOR THE ANALYSIS OF ANIMAL SUBSTANCES.²

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THE proximate analysis of animal substances is as yet confined to the determination of only a few classes of chemical compounds. In continuing the study of the chemistry of the cooking of meats, upon which considerable work has been done in this laboratory in coöperation with the U. S. Department of Agriculture, Office of Experiment Stations,³ it became apparent that the ordinary methods used in the analysis of food materials do not give a sufficient amount of information regarding the proximate constituents which uncooked and cooked meats and broths contain, to teach much regarding the nature of the changes resulting in the processes of cooking.

It is also true that the methods of analysis commonly used do not include the direct determination of several constituents of flesh which are of much importance in imparting to meats their characteristic flavors and which in addition possess real value as stimulants to digestion. These principles are the nitrogenous and non-nitrogenous extractives.

Further, there are also serious objections to the customary methods of analysis which require the preliminary preparation of air-dried samples, since this procedure brings about fundamental

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² This research was made possible through the valuable aid given by the Department of Animal Husbandry of the Agricultural Experiment Station of the University of Illinois.

³ U. S. Department of Agriculture, Office of Experiment Stations, *Bulls.* 102 and 104.

changes in the proteid substances and undoubtedly affects, to some extent, the nature of the fats and probably the organic extractives. The changes which the process of air-drying produces in the proteids prevent further separation, determination and examination of these substances, since they are for the most part, if not entirely, made insoluble by this treatment.

In view of these facts it seemed desirable to attempt, in the first place, to analyze directly the fresh substance of meats without first preparing an air-dried sample, which is the usual practice, and in the second place, to prepare and study water extracts of flesh so that, if possible, a more complete differentiation could be made between their proximate principles.

METHOD FOR THE DIRECT ANALYSIS OF FLESH.

As a result of considerable preliminary experimental work it has been demonstrated that the method recommended by the Association of Official Agricultural Chemists may be so modified that it can be used to analyze directly the fresh substance of meats without the previous preparation of air-dried samples. It will, therefore, only be necessary to describe here such modifications as have been found desirable, in order to use the fresh substance of the food materials.

It is essential that much care be taken in preparing and thoroughly mixing the samples for the analysis. In the case of flesh the samples were ground in a sausage mill and the minced meat was then intimately mixed. This operation of grinding and mixing was performed three or four times for each sample. On account of the large proportion of water in the samples of fresh meats all the weighings were made by difference, a glass-stoppered weighing-bottle being used.

For the determination of water the samples were weighed in glass tubes with filter-paper bottoms, such as are ordinarily used in ether extraction of fat by the Hopkins method.¹ In cases where the meat was rather fat, filter blocks, made of Schleicher & Schüll fat-free paper, were first put into the tubes. The tube and contents were dried very slowly at first in the water-oven at a low temperature. The determination was then continued as usual.

The fat was then determined by extracting the dried samples from the estimation of water with anhydrous ether. After twenty-four hours' extraction the samples were removed and intimately

¹ Ill. Agr. Expt. Sta., Bull. 53, 147 (1898).

ground with ignited sand, then transferred to the moisture tubes again, which were loosely plugged with fat-free cotton to prevent any of the material from being mechanically carried into the flasks. The extraction was then further continued for twelve hours.

The usual Kjeldahl method was used for the determination of the total nitrogen, special care being taken in transferring the weighed meat to the Kjeldahl flask, so that none of the material adhered to the neck of the flask. In the distillation it was found that ignited pumice stone was preferable to granulated zinc. Experiments made in this laboratory show that zinc reduces some form of nitrogen compounds which is usually present in the ordinary alkali and thus introduces a slight error.

The ash was determined as usual, but much care was taken to heat the muffle very slowly and gradually, to begin with, so as to prevent loss by sputtering. The total phosphorus in the meat was determined by the method described by Neumann.¹

In order to test the accuracy of this method a piece of raw, lean, beef sirloin was thoroughly ground and mixed and then divided into two samples, which were given different laboratory numbers. One of these samples was analyzed completely and carefully by one analyst and the other sample was analyzed by another analyst.

It was not known by either of the analysts that the samples were the same. In the earlier work in testing the methods as to accuracy each determination was made in double duplicates. The results of this test of the method are given below.

TABLE I.—RESULTS OF THE ANALYSIS OF THE SAME SAMPLE BY DIFFERENT ANALYSTS.

Laboratory No.	Kind of meat.	Water. Per cent.	Protein (N X 6.25)		Fat. Per cent.	Ash Per cent.	Total nitrogen. Per cent.
			Per cent.	Per cent.			
1649a	Beef sirloin, raw	75.32	21.50	3.27	1.02	3.44	
1649b	"	75.53	20.81	2.70	1.03	3.33	
1649c	"	75.66	21.13	3.07	1.02	3.38	
1649d	"	75.32	21.62	3.27	0.99	3.46	
	Average	75.46	21.27	3.08	1.02	3.40	
1650a	Beef sirloin, raw	75.81	21.25	3.04	1.02	3.40	
1650b	"	75.44	21.31	2.99	0.99	3.41	
1650c	"	75.50	21.31	2.72	1.00	3.45	
1650d	"	... ¹	21.56	... ²	1.02	3.41	
	Average	75.58	21.36	2.92	1.01	3.42	

¹ Dubois Reymond's Archiv. (Physiol. Abth.), 1897, p. 552.

² Determination lost.

These figures indicate very clearly that this method, even when used by different analysts, gives concordant results that are strictly comparable with each other.

However, before accepting this method for use in place of the usual method in which the meats were first air-dried, a comparative test was made of the two methods upon a considerable number of samples. For this purpose each cut of meat was very thoroughly sampled and then divided into two portions—one for the direct analysis in which the fresh sample was used as indicated above, and another for the preparation of an air-dried sample which was then analyzed by the usual method. The results of these comparative analyses are given in Table II. The results for each sample of meat are the average of either triplicate or quadruple determinations.

The examination of the data given in the above table leads to the conclusion that while the agreement of the results is not yet as good as would be desired, they are in sufficiently close agreement to fully justify the use of the modified method, in which the food material is analyzed directly without previous air-drying.

In view of the comparative results thus obtained this method of direct analysis has been used exclusively in this laboratory during the last year in the analysis of a large number of samples of raw and cooked meats.

PREPARATION AND ANALYSIS OF COLD WATER EXTRACTS OF FLESH.

Investigations which have been made in this laboratory led to the conclusion that one of the best means of increasing the present very incomplete knowledge of the chemistry of flesh would be a thorough study of the cold water extracts of the fresh substance of the same.

In the past, comparatively little work has been undertaken in this direction, and as far as the writers have been able to discover, no method has been as yet proposed or described for so complete an examination of the cold water extracts of flesh as the one which is given in this paper. After much preliminary experimentation, which it is not necessary to describe in this connection, the following method for the preparation and analysis of the cold water extracts of meats was elaborated and adopted in this laboratory for use in an extended investigation upon the chemistry of flesh.

Preparation of the Cold Water Extracts.—Three portions of 30 to 35 grams each of the thoroughly sampled meat were taken. The weighings were made by difference, a glass-stoppered weighing-tube being used. The meat was thoroughly mixed each time before taking any of it. Each lot of 30 to 33 grams was divided as equally as possible between six small beakers. The samples were moistened with a little distilled water and all the lumps completely broken up with a glass rod. Fifty cc. of water were then added to each beaker and the contents stirred thoroughly for fifteen minutes. After allowing three to five minutes for the insoluble residue to settle, the liquid for each beaker was decanted through filters into 250-cc. flasks. The insoluble portion was thoroughly drained and then 25 cc. of water were added. The water and residue were thoroughly stirred for seven to eight minutes and after settling decanted upon the same filter. This treatment was continued—using each time 25 cc. of water—until the filtrate measured about 230 cc. The filters were allowed to drain completely between each extraction. After the last extraction the entire contents of each beaker were thrown upon the filter and, when drained, washed twice with a small quantity of distilled water. The filtrates were then made up to 5 liters, after rinsing each flask twice with water.

Completion of the Extraction with Cold Water.—In order to test whether or not the extraction with cold water, as made above, was complete, a second extract of the residues of meat from the first extraction was prepared. This second extraction was made exactly like the first one in every detail. The second cold water extract was diluted to the same volume as the first and it was also analyzed by exactly the same methods.

Further, a third extract was also prepared from a portion of the insoluble residues left from the second extraction, in which only the total solids and ash were determined.

In order to further check the accuracy of the methods for the preparation and analysis of the water extracts of meats, after the second extraction with cold water, a portion of the insoluble residues was used for the determination of total nitrogen, fat and ash. The results of these several experiments are tabulated in the following table.

TABLE III.—RESULTS SHOWING COMPLETION OF EXTRACTION WITH COLD WATER.

Constituents.	Raw meat before extraction.	Cold water extracts.			Insoluble residue from second extraction.	Sum of residue and first and second extractions.
		First extraction.	Second extraction.	Third extraction.		
		Lab. No. 1737. Per cent.	Lab. No. 1738. Per cent.	Lab. No. 1740. Per cent.		
Total solids.....	7.0280	0.1110	0.0910
Total ash.....	1.19	0.8513	none	none	0.1400	1.0913
Total nitrogen.....	3.609	0.8435	0.0148	0.0069	2.7410	3.5993
Nitrogen coagulated by heat.....	0.3925	0.0040
Nitrogen precipitated by ZnSO ₄	0.0473	0.0082
Sum of coagulable and albumose nitrogen..	0.4398	0.0122
Nitrogen precipitated by tannin and salt..	0.4331	0.0071
Non-proteid nitrogen.	0.4037	0.0026
Total fat.....	2.78	2.53	2.53
Water.....	73.23

These results indicate that the method used in preparing the cold water extracts does not give entirely complete extraction. The total solids in the second water extract amounted to only 1.58 per cent. of the total solids in the first cold water extract. The total nitrogen of the second extraction equaled only 0.41 per cent. of the total quantity of nitrogen existing in the first extract. Considering the difficulties attending this work, it seems to the writers that the extraction can be considered practically complete and that the results are quite within the limits of error.

ANALYSIS OF COLD WATER EXTRACTS.

Determination of Total Solids and Ash.—Portions of 100 cc. each of the cold water extract were evaporated to dryness in weighed platinum dishes. The residues were dried in the water-oven for periods of one hour until the weight of each was approximately constant. The dried residues were ignited carefully over the free flame at a low red heat until colorless or nearly so. The ash was weighed quickly, reheated and weighed again. The treatment was repeated until the weight was constant.

Determination of Total Nitrogen.—Portions of 100 cc. each of the extract were used to determine the nitrogen by the usual Kjeldahl method. Dilute standard solutions, about N/15, were used for this part of the work.

Coagulable Proteids.—To begin with, considerable trouble was experienced in this determination in getting even fairly concordant results. It has been found in this work that even a very slight excess of acetic acid interferes materially with the complete separation and proper coagulation of the coagulable proteids occurring in water solutions of raw meats. Professor W. O. Atwater,¹ in the study of the chemical composition of fish, found that the presence of acetic acid instead of increasing the amount of proteid coagulated in a water solution of fresh fish, was very apt to hinder coagulation and sometimes prevent it altogether. In this laboratory it has been found that in the presence of acetic acid the proteid separates as a slimy film upon the surface of the liquid, the coagulation being incomplete and the filtration taking place very slowly. In all cases an additional separation takes place upon further evaporation of the filtrate for the precipitation of albumoses and also upon neutralization. In fact, separation of additional proteid continues until the solution is evaporated to dryness upon the water-bath. This incomplete coagulation resulted even when 2 cc. of N/10 acetic acid was used. Such difficulties were not so apparent in the analysis of cold water extracts from cooked meats, which, however, contained as a rule very slight amounts of coagulable proteids.

In order to overcome these difficulties the coagulation in the latter determinations was brought about in a neutral solution, after some preliminary experiments had proved conclusively that under such conditions the precipitation of coagulable matter was more complete, that the filtration under such conditions always took place readily, and that more concordant results were obtained. It was also proved that the coagulation was more complete when the solutions were made neutral to litmus paper, than it was when the solutions were made neutral to phenolphthalein. In order to test the influence of acids and alkalis upon the coagulation of the proteids contained in the cold water extracts of flesh the following experiment was made: Fifteen portions of a cold water extract of meat, measuring 200 cc. each, were taken.

¹ U. S. Fish Commissioner's Report for 1880, p. 244 (Washington, 1883).

Three of these portions were each treated with 2 cc. of $N/10$ acetic acid. They were all then carefully evaporated upon the water-bath until the volume in each case was reduced to 50 cc. The twelve portions to which no acetic acid had been added coagulated very nicely, while the three fractions which contained acetic acid failed to coagulate, but a slimy film separated upon the surface of the liquids.

After the volume of the solutions had been reduced to 50 cc. the beakers containing the liquids were removed from the water-bath and treated as follows:

(a) Three portions were exactly neutralized with $N/10$ NaOH solution, using litmus as indicator. For this purpose 0.35 cc. of $N/10$ NaOH solution were required.

(b) Three portions were each treated with 0.70 cc. NaOH solution, which made them slightly, but distinctly alkaline to litmus paper.

(c) Three portions were exactly neutralized with $N/10$ NaOH solution, using phenolphthalein as indicator. These solutions required 4.4 cc. of $N/10$ NaOH solution.

(d) Three portions were each treated with 2 cc. of $N/10$ acetic acid solution.

(e) The three portions containing the 2 cc. of acetic acid were not neutralized or changed in any way.

After the above treatments the fifteen portions were all replaced upon the water-bath for fifteen minutes and then filtered immediately while hot. The resulting coagulated precipitates were thoroughly washed with boiling water and the nitrogen determined in them by the Kjeldahl method. The filtrates and washings were all replaced upon the water-bath and then concentrated to 50 cc. No additional precipitates were formed. All solutions were now made exactly neutral to litmus and evaporated to 25 cc. In (a) where the solutions were made neutral to litmus by adding 0.35 cc. of $N/10$ NaOH solution, and in (b) where the solutions were made only slightly alkaline to litmus by the addition of 0.70 cc. of $N/10$ NaOH solution there were no additional precipitates formed by this treatment. In (e) where 2 cc. of acetic acid were added before evaporation, in (c) where the solutions were neutralized with $N/10$ NaOH solution, using phenolphthalein as indicator, and in (d) where the solutions were treated with 2 cc. of $N/10$ acetic acid after evaporation, there were additional

precipitates produced upon neutralization and evaporation. These were separated and washed as above and then the nitrogen determined.

The complete results of these experiments are given in the table on the following page.

These preliminary experiments, together with others which confirmed these results, led to the adoption of the following method for the determination of the coagulable proteids in cold water extracts of flesh: Portions measuring 200 cc. each were evaporated upon the water-bath to a volume of 50 cc. The solutions were then exactly neutralized with N/10 NaOH solution, litmus paper being used as the indicator. The neutral solutions were warmed upon the water-bath for ten minutes and immediately filtered and the coagulated residues washed thoroughly with hot water. The nitrogen in the residue was determined, much care being taken to remove all the coagulated proteid from the beakers by the aid of hot concentrated sulphuric acid.

Albumoses and Proteoses.—For the determination of these substances in the water extracts of meats, the zinc sulphate method proposed by A. Bömer¹ was used. Special precautions were taken in order to have the conditions of precipitation the same as regards temperature, acidity, volume of liquid and time of standing. The details of the method of procedure finally decided upon were as follows: The filtrates and washings from the above determinations of coagulable proteid, after slight acidification with acetic acid, were evaporated upon the water-bath to a volume of 30 cc. and allowed to cool. One cc. of 50 per cent. sulphuric acid was added and the solution completely saturated with crystallized zinc sulphate. The solution was then heated upon the water-bath with constant stirring until perfectly clear, allowed to stand twelve hours, filtered, and the precipitate thoroughly washed with a completely saturated solution of zinc sulphate, slightly acidified with sulphuric acid. The nitrogen in the precipitate was determined by the Kjeldahl method.

Peptones.—Tannic acid has long been recognized as an excellent precipitant for proteid bodies. Almen² probably first used tannin as a quantitative reagent for proteids. The following investigators have made use of this reagent for the estimation and separa-

¹ *Z. anal. Chem.*, 34, 562 (1895).

² *Uppsala lakareforenings forhandlingor* (1870).

TABLE IV.—INFLUENCE OF ACIDITY AND ALKALINITY UPON COAGULATION OF PROTEIDS.

Method of procedure : First experiment.							First test.	Second test.	Third test.	Average.
							Per cent.	Per cent.	Per cent.	Per cent.
(a)	Neutralized with 0.35 cc. of NaOH (litmus)	0.4040	0.4033	0.4038	0.4037
(b)	Made slightly alkaline with 0.7 cc. of NaOH (litmus)	0.4054	0.4049	0.4049	0.4059
(c)	Neutralized with 4.4 cc. of NaOH (phenolphthalein), first precipitate	0.3984	0.3855	0.3989	0.3943
	“ “ “ “ “ “ “ second precipitate	0.0085	0.0096	0.0096	0.0092
	“ “ “ “ “ “ “ total	0.4069	0.3951	0.4085	0.4035
(d)	Acidified with 2 cc. of acid, after evaporation, first precipitate	0.4019	0.3980	0.3924	0.3974
	“ “ “ “ “ “ “ second precipitate	0.0078	0.0051	0.0085	0.0071
	“ “ “ “ “ “ “ total	0.4097	0.4031	0.4009	0.4046
(e)	Acidified with 2.0 cc. of acid before evaporation, first precipitate	0.1237	0.2296	0.2333	0.1955
	“ “ “ “ “ “ “ second precipitate	0.2763	0.1913	0.1665	0.2114
	“ “ “ “ “ “ “ total	0.4000	0.4209	0.3998	0.4069
Method of procedure : Second experiment.										
(a)	Neutralized with 0.25 cc. of NaOH (litmus)	0.4620	0.4565	0.4630	0.4608
(b)	Made slightly alkaline with 0.5 cc. of NaOH (litmus)	0.4651	0.4636	0.4623	0.4637
(c)	Neutralized with 3.9 cc. of NaOH (phenolphthalein) first precipitate	0.4562	0.4484	0.4574	0.4540
	“ “ “ “ “ “ “ second precipitate	0.0110	0.0091	0.0090	0.0097
	“ “ “ “ “ “ “ total	0.4672	0.4575	0.4664	0.4637
(d)	Acidified with 2.0 cc. of acid before evaporation, first precipitate	0.1526	0.0670	0.1469	0.1222
	“ “ “ “ “ “ “ second precipitate	0.3068	0.3869	0.3136	0.3357
	“ “ “ “ “ “ “ total	0.4594	0.4539	0.4605	0.4579

tion of proteid nitrogen from non-proteid nitrogen in the various organic substances which they have studied: Libarius, Girgensohn, Taraszewicz, Sebelien, Mallet, Vivian, Schjerning, Van Slyke and Hart, Bigelow and others. It is still a disputed question as to whether tannin is a complete precipitant for all kinds of proteids, but the literature regarding this matter is too comprehensive to take up in connection with this paper. Tannin and salt have been used in this research for two purposes, first to determine the quantity of peptones, if present, and second, since peptones occur in very small quantity, if at all, in fresh flesh, the results which have been obtained by this precipitant have been used as a check upon the total soluble proteid nitrogen which was obtained by the summation of the nitrogen precipitated by coagulation and by zinc sulphate.

In the first part of this work tannin and sodium chloride were used as a precipitant under the following conditions: Portions of the cold water extracts of raw and cooked meats measuring 200 cc. were transferred into 250-cc. measuring flasks and then treated directly with 1 gram of sodium chloride and 5 cc. of a solution containing 12 per cent. of tannic acid. The solution was then diluted to 250 cc., allowed to stand twelve hours or more, filtered through a dry filter and the nitrogen determined in a measured volume of 200 cc. of the filtrate.

In later work, tannin and salt have been used not directly upon the cold water extracts, but upon the filtrates resulting from the coagulation of the proteids in neutral solutions. In such cases the details of the procedure were as follows: Portions of the cold water extracts of raw and cooked meats measuring 200 cc. were carefully evaporated upon the water-bath until the volume in each case was reduced to 50 cc. The liquids were then exactly neutralized with N/10 NaOH solution, using litmus paper as an indicator, and they were then replaced upon the water-bath for fifteen minutes. The coagulable proteid was then removed immediately by filtration and thoroughly washed with hot water. The filtrates and washings were replaced upon the water-bath, concentrated to about 75 cc. and then transferred to 110-cc. measuring flasks. One gram of salt dissolved in 5 cc. of water and 5 cc. of tannic acid were added. The solution was then diluted to a volume of 110 cc., thoroughly mixed and allowed to stand

over night. The solutions were filtered through dry filters and the nitrogen determined in 100-cc. portions of the filtrates.

The amount of nitrogen in the form of peptones was determined by difference; that is, by subtracting from the nitrogen precipitated by tannin and salt, the combined amount of nitrogen precipitated by coagulation and by zinc sulphate.

Inorganic and Organic Phosphorus.—The total soluble phosphorus was determined in 250-cc. portions of the cold water extract by the Neumann method. The inorganic phosphorus in the cold water extracts of meats was determined in 500-cc. portions of the solution by the method finally adopted by Hart and Andrews¹ with such modifications as it has been found necessary to make in the case of meats. The method now used for the work in this laboratory is as follows: The 500-cc. portions are evaporated to about 100 cc. and filtered to remove the coagulated proteid. The filtrates are made neutral to litmus paper by ammonium hydroxide and about 8 grams of ammonium nitrate added to the same. The solutions are then warmed to 60° C., and 3 cc. of nitric acid (sp. gr. 1.20) are added. The inorganic phosphorus is then precipitated by an excess of a clear, neutral solution of ammonium molybdate. After the addition of the precipitant the solution is digested for fifteen minutes at a temperature of 60° C., and then allowed to stand at the temperature of the laboratory for two hours. After filtration, the precipitate is dissolved in ammonium hydroxide and then reprecipitated by acid ammonium molybdate and the determination continued as usual. The phosphorus thus precipitated was considered to be that due to inorganic phosphates.

In connection with the study of the cold water extracts of flesh a somewhat extended research was made to determine the relative value of the following reagents for the separation and estimation of proteid and non-proteid nitrogen: Phosphotungstic acid in the cold, phosphotungstic acid in the hot, bromine, and Stützer's reagent. The results of this work will be published in a later paper.

Calculation of the Final Results of Analysis.—By the direct analysis of the fresh meats the following data are obtained: The percentage of water, total nitrogen, fat, ash and total phosphorus. From the results of the analysis of the cold water extract of the

¹ *Am. Chem. J.*, 30, 470.

meats the following data are directly obtained: The percentage of soluble ash, soluble nitrogen, soluble nitrogen coagulable by heat, albumose nitrogen, peptone nitrogen, total soluble phosphorus, soluble inorganic phosphorus and total soluble matter.

From the two sets of analytical data mentioned above which are obtained by direct determination, it is possible to represent the composition of the flesh in the following terms: Soluble coagulable proteid, albumose, peptone, total soluble proteid, insoluble proteid, total proteid, nitrogenous extractives, non-nitrogenous extractives, total organic extractives, fat, soluble ash, insoluble ash, total ash, soluble inorganic phosphorus, soluble organic phosphorus, total soluble phosphorus, insoluble phosphorus and total phosphorus.

The soluble coagulable proteid, albumose and peptone are obtained by multiplying the nitrogen existing in these forms by the factor 6.25. The total soluble proteid is equivalent to the sum of these last three quantities. The insoluble proteid is obtained by subtracting the total soluble nitrogen from the total nitrogen in the meat and then multiplying this difference by the factor 6.25. The total proteid is obtained by the summation of the soluble and the insoluble proteid.

The percentage of the nitrogenous organic extractives is calculated by subtracting the soluble proteid nitrogen from the total soluble nitrogen and then multiplying this difference by the factor 3.12. The non-nitrogenous extractives are found by subtracting the sum of the total soluble proteid, the nitrogenous extractives and the soluble ash from the total soluble material found by direct determination. The total organic extractives represent the sum of the nitrogenous and non-nitrogenous extractives.

The fat and total ash are determined directly in the fresh substance of the flesh. The soluble ash is found directly in the cold water extract. The insoluble ash is obtained by difference.

The total phosphorus, the total soluble phosphorus and the soluble inorganic phosphorus are determined as indicated above. The soluble organic phosphorus and the insoluble phosphorus are obtained by difference.

The representative results given in the following tables indicate the extent and character of the information furnished by use of the improved methods of analysis herewith reported, as compared with the results obtained by the ordinary method of analysis.

TABLE V.—CHEMICAL COMPOSITION OF MEATS BY THE IMPROVED METHODS OF ANALYSIS—FRESH SUBSTANCE.

Laboratory No.	Kind of meat.	Proteid.													Fat. Per cent.
		Dry substance.			Soluble.						Organic extractives.				
		Water. Per cent.	Soluble. Per cent.	In-soluble. Per cent.	Total. Per cent.	Coag- ulable. Per cent.	Albu- mose. Per cent.	Pept- ones. Per cent.	Total. Per cent.	In- soluble. Per cent.	Total. Per cent.	Nitroge- nous. Per cent.	nitroge- nous. Per cent.	Total. Per cent.	
1850	Beef, round, raw . . .	74.89	7.26	18.00	25.26	2.62	0.23	0.08	2.93	15.67	18.60	1.34	2.00	3.34	2.24
1823	Beef, round, raw . . .	75.61	6.21	18.19	24.40	2.08	0.23	0.10	2.41	15.97	18.38	1.26	1.54	2.80	2.14
1857	Veal, breast, raw . . .	63.34	4.65	31.86	36.51	1.55	0.18	0.06	1.79	15.19	16.89	0.78	1.42	2.20	16.52
1860	Veal, leg, raw	73.40	5.81	21.50	27.31	1.52	0.22	0.10	1.84	16.28	18.12	1.21	1.87	3.08	5.02
1831	Beef, ribs, roast . . .	44.78	2.76	52.32	55.08	0.36	0.14	0.02	0.52	15.30	15.82	0.77	0.82	1.59	36.84
1838	Beef, ribs, roast . . .	49.73	3.04	47.63	50.67	0.26	0.12	0.00	0.38	19.65	20.03	0.91	1.04	1.95	27.79
1829	Beef, round, pot-roast	57.57	3.24	39.61	42.85	0.00	0.43	0.03	0.46	35.14	35.60	1.01	1.09	2.10	4.20
1825	Beef, round, pot-roast	56.92	4.24	40.11	44.35	0.00	0.32	0.05	0.37	34.32	34.69	1.31	1.53	2.84	5.58
1824	Beef, round, boiled . .	55.96	2.92	41.69	44.61	0.00	0.39	0.02	0.41	33.01	33.42	0.83	1.06	1.89	8.47
1868	Beef, round, boiled . .	61.70	1.76	37.24	39.00	0.00	0.19	0.14	0.33	33.45	33.78	0.47	0.54	1.01	3.65

Laboratory No.	Kind of meat.	Nitrogen.											Phosphorus.			
		Ash.			Soluble.				Ratio of non-proteid to proteid.				Soluble.			
		Solu- ble. Per cent.	Insolu- ble. Per cent.	Total. Per cent.	Pro- teid. Per cent.	Non- pro- teid. Per cent.	Total. Per cent.	Insolu- ble. Per cent.	Total. Per cent.	In water. Ratio.	In meats. Ratio.	Inor- ganic. Per cent.	Or- ganic. Per cent.	Total. Per cent.	Insolu- ble. Per cent.	Total. Per cent.
1850	Beef, round, raw . . .	0.99	0.99	1.08	0.469	0.431	0.900	2.506	3.406	1 : 1.09	1 : 7.91	0.153	0.104	0.257	0.688	0.345
1823	Beef, round, raw . . .	1.00	0.08	1.08	0.386	0.402	0.788	2.556	3.344	1 : 0.96	1 : 8.32	0.090	0.097	0.187	0.035	0.222
1857	Veal, breast, raw . . .	0.66	0.24	0.90	0.286	0.251	0.537	2.416	2.953	1 : 1.14	1 : 11.76	0.075	0.037	0.112	0.087	0.199
1860	Veal, leg, raw	0.89	0.20	1.09	0.292	0.387	0.679	2.665	3.284	1 : 0.75	1 : 8.49	0.118	0.039	0.157	0.064	0.221
1831	Beef, ribs, roast . . .	0.65	0.18	0.83	0.082	0.248	0.330	2.449	2.779	1 : 0.33	1 : 11.21	0.095	0.023	0.118	0.030	0.148
1838	Beef, ribs, roast . . .	0.71	0.19	0.90	0.060	0.292	0.352	3.144	3.496	1 : 0.21	1 : 11.97	0.104	0.044	0.148	0.042	0.190
1829	Beef, round, pot-roast	0.68	0.27	0.95	0.073	0.322	0.395	5.623	6.018	1 : 0.23	1 : 18.70	0.112	0.049	0.161	0.066	0.227
1825	Beef, round, pot-roast	1.03	0.21	1.24	0.059	0.419	0.478	5.491	5.969	1 : 0.14	1 : 14.24	0.122	0.059	0.181	0.083	0.264
1824	Beef, round, boiled . .	0.62	0.21	0.83	0.039	0.266	0.305	5.396	5.611	1 : 0.15	1 : 21.10	0.085	0.04	0.126	0.087	0.213
1868	Beef, round, boiled . .	0.42	0.14	0.56	0.053	0.151	0.204	5.351	5.555	1 : 0.35	1 : 36.79			0.068	0.094	0.162

TABLE VI.—CHEMICAL COMPOSITION OF MEATS BY THE ORDINARY METHOD OF ANALYSIS—FRESH SUBSTANCE.

Laboratory No.	Kind of meat.	Water. Per cent.	Dry substance. (N \times 6.25). Per cent.	Proteid Per cent.	Fat. Per cent.	Ash. Per cent.	Total nitrogen. Per cent.
1850	Beef, round, raw.....	74.89	24.61	21.29	2.24	1.08	3.406
1823	Beef, round, raw.....	75.61	24.13	20.91	2.14	1.08	3.345
1857	Veal, breast, raw.....	63.34	35.88	18.46	16.52	0.90	2.953
1860	Veal, leg, raw.....	73.40	26.64	20.53	5.02	1.09	3.284
1831	Beef, ribs, roast.....	44.78	55.04	17.37	36.84	0.83	2.779
1838	Beef, ribs, roast.....	49.73	50.54	21.85	27.79	0.90	3.496
1829	Beef, round, pot-roast...	57.57	42.76	37.61	4.20	0.95	6.018
1825	Beef, round, pot-roast...	56.92	44.13	37.31	5.58	1.24	5.969
1824	Beef, round, boiled.....	55.96	44.37	35.07	8.47	0.83	5.611
1808	Beef, round, boiled.....	61.70	38.93	34.72	3.65	0.56	5.555

For Table VII see next page.

TABLE VIII.—CHEMICAL COMPOSITION OF MEATS BY THE ORDINARY METHOD OF ANALYSIS—WATER-FREE SUBSTANCE.

Laboratory No.	Kind of meat.	Dry substance. (N \times 6.25). Per cent.	Proteid Per cent.	Fat. Per cent.	Ash. Per cent.	Total nitrogen. Per cent.
1850	Beef, round, raw.....	100.00	86.51	9.10	4.39	13.840
1823	Beef, round, raw.....	100.00	86.66	8.87	4.47	13.862
1857	Veal, breast, raw.....	100.00	51.45	46.04	2.51	8.232
1860	Veal, leg, raw.....	100.00	77.07	18.84	4.09	12.327
1831	Beef, ribs, roast.....	100.00	31.56	66.93	1.51	5.049
1838	Beef, ribs, roast.....	100.00	43.23	54.99	1.78	6.917
1829	Beef, round, pot-roast...	100.00	87.96	9.82	2.22	14.074
1825	Beef, round, pot-roast...	100.00	84.55	12.64	2.81	13.526
1824	Beef, round, boiled.....	100.00	79.04	19.09	1.87	12.646
1808	Beef, round, boiled.....	100.00	89.19	9.37	1.44	14.269

TABLE IX.—CHEMICAL COMPOSITION OF MEATS BY THE ORDINARY METHOD OF ANALYSIS—WATER-FREE AND FAT-FREE SUBSTANCE.

Laboratory No.	Kind of meat.	Fat-free dry substance. Per cent.	Proteid. Per cent.	Ash. Per cent.	Total nitrogen. Per cent.
1850	Beef, round, raw.....	100.00	95.17	4.83	15.225
1823	Beef, round, raw.....	100.00	95.09	4.91	15.211
1857	Veal, breast, raw.....	100.00	95.35	4.65	15.256
1860	Veal, leg, raw.....	100.00	95.00	5.00	15.189
1831	Beef, ribs, roast.....	100.00	95.44	4.56	15.269
1838	Beef, ribs, roast.....	100.00	96.05	3.95	15.367
1829	Beef, round, pot-roast...	100.00	97.54	2.46	15.607
1825	Beef, round, pot-roast...	100.00	96.80	3.20	15.488
1824	Beef, round, boiled.....	100.00	97.69	2.31	15.627
1808	Beef, round, boiled.....	100.00	98.42	1.58	15.745

TABLE VII.—CHEMICAL COMPOSITION OF MEATS BY THE IMPROVED METHODS OF ANALYSIS—WATER-FREE SUBSTANCE.

Laboratory No.	Kind of meat.	Proteid.											
		Dry substance.			Soluble.					Organic extractives.			
		Soluble. Per cent.	Insoluble. Per cent.	Total. Per cent.	Coagulable. Per cent.	Albumenose. Per cent.	Pep-tones. Per cent.	Total. Per cent.	Insoluble. Per cent.	Total. Per cent.	Nitrogenous. Per cent.	Non-nitrogenous. Per cent.	Total. Per cent.
1850	Beef, round, raw	28.74	71.26	100.00	10.37	0.91	0.32	11.60	62.03	73.63	5.30	7.92	13.22
1823	Beef, round, raw	25.45	74.55	100.00	8.53	0.94	0.41	9.88	65.45	75.33	5.16	6.31	11.47
1857	Veal, breast, raw	12.74	87.26	100.00	4.24	0.50	0.16	4.90	41.36	46.26	2.14	3.89	6.03
1860	Veal, leg, raw	21.27	78.73	100.00	5.37	0.81	0.36	6.74	59.61	66.35	4.43	6.86	11.29
1831	Beef, ribs, roast	5.01	94.99	100.00	0.65	0.25	0.04	0.94	27.78	28.72	1.40	1.49	2.89
1838	Beef, ribs, roast	6.00	94.00	100.00	0.51	0.24	0.00	0.75	38.78	39.53	1.80	2.05	3.85
1829	Beef, round, pot-roast . .	7.56	92.44	100.00	0.00	1.00	0.07	1.07	82.01	83.08	2.36	2.54	4.90
1825	Beef, round, pot-roast . .	9.56	90.44	100.00	0.00	0.72	0.11	0.83	77.39	78.22	2.95	3.45	6.40
1824	Beef, round, boiled . . .	6.55	93.45	100.00	0.00	0.86	0.04	0.92	74.01	74.91	1.86	2.38	4.24
1808	Beef, round, boiled . . .	4.51	95.49	100.00	0.00	0.49	0.36	0.85	85.77	86.62	1.21	1.38	2.59

Laboratory No.	Kind of meat.	Fat. Per cent.	Nitrogen.											Phosphorus.			
			Ash.			Soluble.					Insoluble.			Soluble.		Insoluble. Per cent.	Total. Per cent.
			Soluble. Per cent.	Insoluble. Per cent.	Total. Per cent.	Pro-teid. Per cent.	Non-pro-teid. Per cent.	Total. Per cent.	Insoluble. Per cent.	Total. Per cent.	Inor-ganic. Per cent.	Or-ganic. Per cent.					
1850	Beef, round, raw	8.87	3.92	0.36	4.28	1.857	1.706	3.563	9.920	13.483	0.606	0.412	1.018	0.348	1.366		
1823	Beef, round, raw	8.77	4.10	0.32	4.42	1.583	1.647	3.230	10.475	13.795	0.369	0.397	0.766	0.144	0.910		
1857	Veal, breast, raw	45.25	1.81	0.66	2.47	0.783	0.687	1.471	6.617	8.088	0.205	0.101	0.306	0.239	0.545		
1860	Veal, leg, raw	18.40	3.26	0.73	3.99	1.069	1.417	2.486	9.546	12.032	0.432	0.143	0.575	0.234	0.809		
1831	Beef, ribs, roast	66.88	1.18	0.33	1.51	0.150	0.449	0.599	4.446	5.045	0.172	0.042	0.214	0.055	0.269		
1838	Beef, ribs, roast	54.85	1.40	0.38	1.78	0.119	0.576	0.695	6.205	6.900	0.205	0.087	0.292	0.083	0.375		
1829	Beef, round, pot-roast . .	9.80	1.58	0.64	2.22	0.171	0.752	0.923	13.121	14.044	0.262	0.114	0.376	0.154	0.530		
1825	Beef, round, pot-roast . .	12.58	2.32	0.47	2.79	0.134	0.945	1.079	12.380	13.459	0.275	0.133	0.408	0.187	0.595		
1824	Beef, round, boiled . . .	18.99	1.39	0.47	1.86	0.088	0.596	0.684	11.894	12.578	0.191	0.092	0.283	0.195	0.478		
1808	Beef, round, boiled . . .	9.36	1.08	0.36	1.44	0.137	0.386	0.523	13.721	14.244	0.174	0.241	0.415		

TABLE X.—CHEMICAL COMPOSITION OF MEATS BY THE IMPROVED METHODS OF ANALYSIS WATER-FREE AND FAT-FREE SUBSTANCE.

Laboratory No.	Kind of meat.	Proteid.											
		Fat-free dry substance.			Soluble.				Organic extractives.				
		Soluble. Per cent.	Insoluble. Per cent.	Total. Per cent.	Coagu- lable. Per cent.	Albu- mose. Per cent.	Pep- tones. Per cent.	Total. Per cent.	Insolu- ble. Per cent.	Total. Per cent.	Nitroge- nous. Per cent.	Non- nitroge- nous. Per cent.	Total. Per cent.
1850	Beef, round, raw	31.54	68.46	100.00	11.38	1.00	0.35	12.73	68.07	80.80	5.82	8.69	14.51
1823	Beef, round, raw	27.90	72.10	100.00	9.35	1.03	0.45	10.83	71.74	82.57	5.65	6.92	12.57
1857	Veal, breast, raw	23.27	76.73	100.00	7.75	0.91	0.29	8.95	75.54	84.49	3.91	7.10	11.01
1860	Veal, leg, raw	26.10	73.91	100.00	6.83	0.99	0.44	8.26	73.05	81.31	5.43	8.41	13.84
1831	Beef, ribs, roast	15.13	84.87	100.00	1.96	0.75	0.12	2.83	83.88	86.71	4.23	4.50	8.73
1838	Beef, ribs, roast	13.29	86.71	100.00	1.13	0.53	0.00	1.66	85.89	87.55	3.99	4.54	8.53
1829	Beef, round, pot-roast . .	8.38	91.62	100.00	0.00	1.11	0.08	1.19	90.92	92.11	2.62	2.81	5.43
1825	Beef, round, pot-roast . .	10.94	89.06	100.00	0.00	0.82	0.13	0.95	88.53	89.48	3.37	3.95	7.32
1824	Beef, round, boiled	8.09	91.91	100.00	0.00	1.06	0.05	1.11	91.36	92.47	2.29	2.94	5.23
1808	Beef, round, boiled	4.98	95.02	100.00	0.00	0.54	0.40	0.94	94.63	95.57	1.33	1.52	2.85

Laboratory No.	Kind of meat.	Nitrogen.											Phosphorus.			
		Ash.			Soluble.				Insoluble.				Soluble.		Insoluble.	
		Soluble. Per cent.	Insoluble. Per cent.	Total. Per cent.	Proteid. Per cent.	Non- proteid. Per cent.	Total. Per cent.	Insolu- ble. Per cent.	Total. Per cent.	Inor- ganic. Per cent.	Or- ganic. Per cent.	Total. Per cent.	Insolu- ble. Per cent.	Total. Per cent.		
1850	Beef, round, raw	4.30	0.39	4.69	2.038	1.872	3.910	10.885	14.795	0.665	0.452	1.117	0.382	1.499		
1823	Beef, round, raw	4.49	0.35	4.84	1.735	1.805	3.541	11.477	15.020	0.404	0.435	0.839	0.157	0.996		
1857	Veal, breast, raw	3.31	1.20	4.51	1.430	1.255	2.687	12.104	14.772	0.374	0.185	0.559	0.436	0.995		
1860	Veal, leg, raw	4.00	0.89	4.89	1.308	1.737	3.045	11.700	14.745	0.529	0.175	0.704	0.287	0.991		
1831	Beef, ribs, roast	3.56	1.00	4.56	0.453	1.356	1.809	13.424	15.232	0.521	0.126	0.647	0.164	0.811		
1838	Beef, ribs, roast	3.10	0.84	3.94	0.264	1.276	1.540	13.743	15.283	0.455	0.192	0.647	0.183	0.830		
1829	Beef, round, pot-roast . .	1.75	0.71	2.46	0.190	0.833	1.023	14.546	15.569	0.290	0.127	0.417	0.170	0.587		
1825	Beef, round, pot-roast . .	2.66	0.54	3.20	0.153	1.081	1.234	14.162	15.498	0.315	0.152	0.467	0.214	0.681		
1824	Beef, round, boiled	1.72	0.58	2.30	0.179	0.736	0.915	14.615	15.530	0.235	0.114	0.349	0.241	0.590		
1808	Beef, round, boiled	1.19	0.40	1.59	0.151	0.426	0.577	15.138	15.715	0.192	0.266	0.458		

DISCUSSION OF RESULTS.

It is quite evident from the analytical data presented in the above tables that the modified method of the chemical analyses gives a much more comprehensive knowledge of the chemical composition of flesh than does the ordinary method of analysis.

In the first place, the improved method of analysis shows that a considerable proportion of the dry substance of either the raw or cooked meats is soluble in cold water. From one-fourth to one-third of the total dry material of lean raw meats is soluble under these conditions, while a smaller proportion of fat meats and cooked meats is soluble.

In the second place, the results obtained by the methods of analysis here suggested demonstrate the fact that the proteids contained in flesh differ in character and properties. A large proportion of the proteid material of raw flesh is insoluble in water, but there is a considerable part of the same soluble in cold water. Of the soluble proteids of raw meat the greater quantity is coagulable by heat. However, in addition to the coagulable proteids the cold water extracts of raw flesh contain albumoses which are precipitated by zinc sulphate, and also, apparently slight amounts of peptones which are not precipitated by coagulation or by saturation with zinc sulphate, but which are precipitated by tannin and salt. It is more than probable, however, that this apparently small quantity of peptones is due to the precipitation of a small part of the nitrogenous extractives by the tannin and salt. The amount and character of the proteids of cooked meats vary with the methods of cooking, but in all cases the percentage of the soluble proteids is less and the percentage of the insoluble proteids is greater than it is in uncooked meats.

In the third place, the data here presented prove that meats contain a considerable quantity of the so-called organic extractives. This method of analysis indicates clearly that these organic extractives are not entirely nitrogenous in nature, but that they contain also non-nitrogenous substances. In fresh, lean beef the percentage of nitrogenous organic extractives varies from 1.00 to 1.75, and the non-nitrogenous organic extractives vary from 1.40 to 2.20. The amount of these organic extractives in cooked meats depends upon the method of cooking.

The usual method of calculating the percentage of proteid in

meats by multiplying the total nitrogen by the factor 6.25, evidently gives a much higher figure for the true proteids than should be assigned to them. For example, if the total nitrogen (3.406 per cent.) in beef round, laboratory No. 1850 (Table VI, p. 673) is multiplied by the factor 6.25 there is obtained the number 21.29, which represents the per cent. of proteid as obtained by the usual method of analysis. The total proteid obtained by actual analysis by the methods used in this investigation is only 18.60 per cent. (Table V, p. 672). This gives a difference of 2.69 per cent. Since the nutritive value of the organic extractives is certainly much less than that of proteids, the usual method of analysis and calculation must necessarily lead to erroneous results in reporting and considering the nutritive worth of meats and meat products.

The improved methods of analysis give the same information regarding the water and fat contents of meat as does the usual method. The former method enables the analyst to determine the soluble and insoluble mineral constituents of flesh.

Recently the methods of analysis here proposed have been extended so as to include the determination of the various forms in which the element phosphorus exists in meat. The data here presented are not sufficient to enter into a discussion regarding the amount and distribution of the different forms of phosphorus compounds in flesh. An extended study of the phosphorus contents of flesh is now well under way in this laboratory and the results of this research will be published in the near future.

In the fourth place, the methods of analysis here suggested show that marked differences exist in the chemical composition, between raw meats on the one hand and cooked meats on the other hand. By the use of this method of analysis it has been demonstrated that meats cooked by different methods possess quite a difference in chemical composition.

These facts are of practical importance, since they indicate plainly the nature of the chemical reactions and changes involved in the processes of the cooking of meats.

It is not the object of this paper to discuss this last-mentioned subject, but that the above statements are true, it can readily be seen by a brief study of the data presented in Table X, in which the chemical composition of raw and cooked meats is given, expressed in percentage of the water-free and fat-free substance. It is evident from the data here presented that the fat-free substance

of uncooked meats is much more soluble in cold water than is the fat-free material of cooked meats. It is also apparent that the soluble coagulable proteid, the total organic extractives, the ash and the phosphorus compounds exist in cooked meats in much smaller quantities than they do in uncooked meats. Further, the data here presented show clearly that there are marked differences in the chemical composition of meats cooked by roasting, pot-roasting and boiling.

The more complete information obtained by the application of these methods of analysis, regarding the chemical composition of meats, should aid in throwing considerable additional light upon the nutritive value and upon the ease and rapidity of the digestion and assimilation of different kinds of meat and of meats cooked by different methods.

If it is possible, as the writers fully believe it is, to apply this method of analysis with accuracy to the quantitative examination of meats and other food products, this fact should induce and encourage still further investigations to determine the comparative nutritive value between the soluble and the insoluble proteids, between the different forms of soluble proteids, and also, to study still further the functions which the nitrogenous and the non-nitrogenous organic extractives play in the processes of nutrition.

For the data given in the above tables only a few analyses were selected which represent the application of the methods herewith presented. In connection with the researches which are being made in this laboratory upon the chemistry of flesh, this method has already been used in the analysis of 150 samples of different kinds of raw and cooked meats, and other meat products. The detailed results of these analyses will be published in the near future.

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