The samples sent to the Nutrition Laboratory for analysis were taken from the old crop and packed for shipment during the hot, dry season. Captain McCay also analyzed samples of these food materials from the same crop, but the samples were selected earlier in the year when the crop first came upon the market. The results are as yet unpublished but Captain McCay reports that his analyses agree very satisfactorily with those made by us, allowing for the variations in moisture content occasioned by the difference in time of sampling.

Of special interest in the results here shown is the large amount of fat in the wheat ata. The proportion of fat in wheat flour, as commonly used in this country and as shown by some 200 analyses,[1] is from 0.3 to 1.9 per cent., while for the two samples of wheat ata analyzed by us, it was 3.39 and 2.14 per cent. respectively, and 2.90 per cent. as shown by the Calcutta analyses given in the table. The higher percentage of fat will result in a corresponding increase in the heat of combustion. The proportion of fat in the Gram dhall is also high as compared with the other varieties of dhall, being fully three times that shown for any of the others, and the heats of combustion are likewise higher.

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AN APPLICATION OF THE FOLIN METHOD TO THE DETERMINATION OF THE AMMONIACAL NITROGEN IN MEAT.

BY M. E. PENNINGTON AND A. D. GREENLEE.

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The quantity of loosely bound nitrogen which occurs in protein-rich tissues such as meat has long been recognized as an indication of the freshness of the substance. Bacteria have been recognized as energetic agents in the decomposition of these protein-rich tissues, with the formation of relatively large amounts of such loosely bound nitrogen.

The methods for the estimation of the quantity of this nitrogen have been far from satisfactory, though the Folin method has served well for its estimation in such substances as urine.

For the estimation of the so-called ammoniacal nitrogen in meat and meat extracts, distillation with magnesium oxide has been the method most used.[2] This method, however, is unsatisfactory in that, even after hours of distillation, small quantities of ammoniacal nitrogen continue to be evolved and the conditions of the experiment, especially the rate of distillation, markedly affect the amount of nitrogen split off. That

[1] Atwater and Bryant, *Loc. cit.*, p. 58.

[2] Richardson, THIS JOURNAL, **30**, 1515.

which is evolved during the first hour, when the rate is 200 cc. of distillate, is usually considerably greater than during subsequent hours, though the succeeding periods—even to the fifth or sixth—yield quantities too large to be disregarded. Such being the case, it would seem probable that the boiling with even a mild alkali causes a progressive splitting of more firmly bound nitrogen compounds that would tend to vitiate the results, because the separation of amine or other forms of nitrogen is apparently gradual rather than sharply marked.

Richardson gives successive distillations, up to ten, on hashed frozen meat—presumably beef—showing that approximately one-third of the total nitrogen obtained is evolved in the first hour, and that after the sixth hour the quantities vary from 0.005 to 0.002 per cent. At no time did he get nitrogen-free distillates. For practical purposes he distils but one hour.

The following table indicates the rate of the evolution of nitrogen by the magnesium oxide distillation method, using 25 grams of chicken meat, 10 grams of magnesium oxide and 450 cc. of water, and distilling at such speed that 200 cc. of distillate collect in one hour. 0.1 *N* sulphuric acid is used to collect the free ammonia.

TABLE I.—RATE OF EVOLUTION OF AMMONIACAL NITROGEN BY THE MAGNESIUM OXIDE METHOD.

Description of sample.	Distillation periods, one hour each.	Percentage of nitrogen evolved.
No. 299—Fowls of known history in storage two years. In excellent condition.	1	0.036
	2	0.013
	3	0.011
	Total,	0.060
No. 305—Fowl chilled for 48 hours after slaughter at 32° F., then kept at temperature of cool room for 48 hours.	1	0.033
	2	0.020
	3	0.016
	4	.....
	5	0.009
	Total,	.....
No. 308—Fowl chilled for 24 hours after slaughter at 32° F.	1	0.041
	2	0.019
	3	0.015
	4	0.016
	Total,	0.091
No. 304—Fowls of unknown history in storage 6 years.	1	0.109
	2	0.021
	3	0.017
	4	0.015
	5	0.012
	Total,	0.174
No. 306—Fowl chilled for 48 hours after slaughter at 32° F. Kept at temperature of cool room for 72 hours.	1	0.055
	2	0.023
	3	0.018
	4	0.018
	5	0.019
	6	0.016
	Total,	0.149

From the foregoing determination of ammoniacal nitrogen in the flesh of chickens of varying history it would appear that it gives even less satisfactory results by distillation with magnesium oxide than does beef.

Because the determination of this loosely bound nitrogen should be one of the best methods we have for the detection of deterioration in flesh, there has been an endeavor to adapt the method of Folin to such substances, believing that in the cold and with a weak alkali, such as magnesium oxide, there would be a better chance of a sharp separation of ammoniacal nitrogen.

A study of the literature pertaining to the Folin method has shown a scarcity of detail concerning the time required and the strength of the air current. Folin in his original paper[1] states that for urine analysis 600–700 liters of air per hour are required during a period of 1 to $1^1/_2$ hours. Boussingault in 1850[2] used 56 liters of air per hour for five hours, and found that nitrogen remained. He, therefore, abandoned the method.

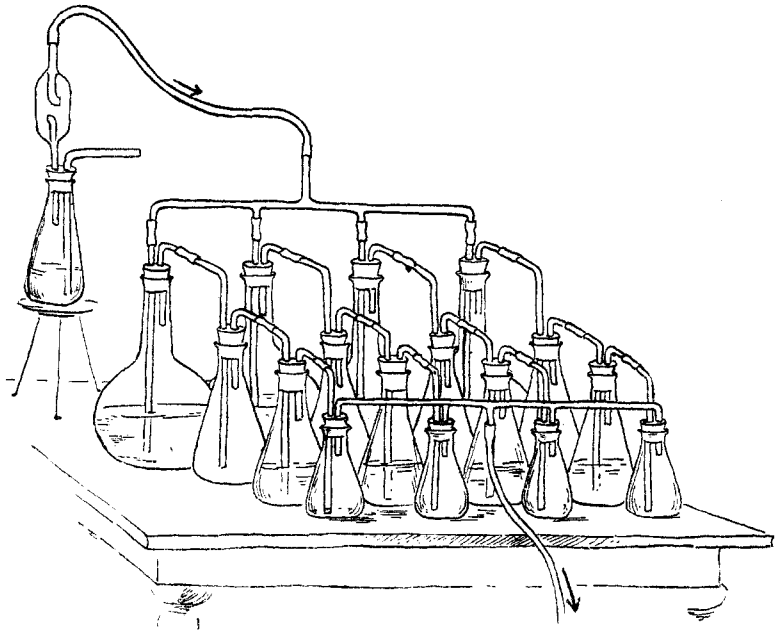
Most of the more recent papers, as those of Kober,[3] Gill and Grindley,[4] and Sebellen, Brymeldsen and Haavardsholm,[5] deal with the conditions necessary for the extraction of the ammonium salts from the Kjeldahl digestion rather than from the original tissue or extract.

It seemed desirable, therefore, to attempt the elimination of loosely bound nitrogen from such substances as meat and its extracts by a strong current of air, and to observe the results between the volume of air and the time required for the doing of the work. It has been found that the usual water pump of the laboratory is not adequate. A small air pump, driven by a $^1/_4$ h. p. motor, answers the purpose, and an anemometer, such as is used to measure the volume of air entering mine shafts, etc., records the total volume of air passing through the apparatus. The ingoing air is purified by sulphuric acid in a flask provided with a Hopkins safety bulb. It then passes through a liter flask in which 25 grams of finely-ground meat are suspended in 250 cc. of water, with 5 grams of magnesium oxide. Between this flask and the 0.1 *N* sulphuric acid absorption bottle is an empty 250 cc. flask, to catch any mechanical spattering or frothing; and beyond the acid bottle a 100 cc. flask is placed to catch any particles of the acid that may pass into the exit tube. The flasks are set up in batteries of four, using one brass or glass four-way tube to connect with the air pump, and another of similar form leading to the air wash bottle (see figure on next page).

It has been found that about 30,000 cubic feet of air through the whole

[1] *Z. physiol. Chem.*, **37**, 161.
[2] *Memoires de Chemie agricole*, 291.
[3] THIS JOURNAL, **30**, 1131.
[4] *Ibid.*, **31**, 1249.
[5] *Chem.-Ztg.*, **33**, 793.

system or 8,000 cubic feet through each individual series of flasks, and a period of three to six hours, is sufficient to remove all of the nitrogen which is eliminated by weak alkali at an ordinary temperature. When such an



elimination has occurred the receiving flasks remain free from ammoniacal nitrogen even though a rapid current of air be passed for several additional hours.

The addition of about 25 cc. of alcohol largely prevents the foaming of the protein, which may happen during the latter part of the aspiration period. When once started but little attention is required except an occasional shaking of the flasks. One gram of sodium fluoride, and a temperature of 10–15°, has been found a satisfactory method of preserving unchanged the suspension of meat in water for a period of 24 hours—a fact which is of considerable importance, if many samples are received at one time.

Like the evolution by distillation with magnesium oxide, the preponderance of ammonia is given off during the first hour but, unlike that procedure, there is a steady decrease until the zero point is reached. Table II illustrates the rate of evolution as influenced by the time and the amount of air.

In certain experiments sodium carbonate was used as a hydrolyzing alkali in quantities varying from 0.5 of a gram to 2 grams, side by side with magnesium oxide. It is of interest to note that the evolution of

TABLE II.—RATE OF EVOLUTION OF AMMONIACAL NITROGEN AS DEPENDENT UPON THE QUANTITY OF AIR AND ITS VELOCITY.

Description of sample.	Extraction period.	Percentage of ammoniacal nitrogen evolved.	Cu. ft. of air used.	Time consumed (in hours).
No. 250–A—Fowl in chill room at 32° F. for 24 hours after slaughter.	1	0.0078	1,668	1
	2	0.0034	1,883	1.16
	3	0.0005	1,150	0.83
	4	0.0003	1,235	1
	5	0.0000	1,435	1
	Total,	0.012	7,371	5
No. 250–B — Fowl in chill room at 32° F. for 24 hours after slaughter. Then the meat was ground fine and preserved in a 0.4 per cent. solution of sodium fluoride for 24 hours.	1	0.0025	366	0.75
	2	0.0031	439	0.75
	3	0.0029	460	0.75
	4	0.0015	480	0.75
	5	0.0011	710	0.75
	6	0.0006	525	0.75
	7	0.0004	360	0.75
	8	0.0002	395	0.75
	9	0.0000	415	0.75
	Total,	0.012	4,150	6.75
No. 251–A—Fowl kept in a cool room for six days.	1	0.0095	3,805	1.5
	2	0.0029	2,308	1
	3	0.0014	2,750	1
	4	0.0007	3,670	1
	5	0.0000	3,180	1
	Total,	0.015	15,713	5.5
No. 252—Fowl of known history, two years in storage. Badly gnawed by rats.	1	0.0235	2,600	1
	2	0.0070	3,009	1
	3	0.0039	3,025	1
	4	0.0011	3,175	1
	5	0.0006	3,120	1
	6	0.0000	1,660	1
	Total,	0.036	16,580	6
No. 257–A—Fowl of known history, two years in storage. In fair condition.	1	0.0224	3,150	1.16
	2	0.0044	2,800	1.16
	3	0.0011	2,430	1
	4	0.0000	2,750	1.16
	Total,	0.028	11,130	4.48

ammoniacal nitrogen was the same with both reagents. Such findings may be taken as evidence of the sharply differentiated linkage of loosely bound nitrogen in protein, which is certainly increased during bacterial decomposition and probably during enzyme decomposition (Table III).

TABLE III.—EVOLUTION OF AMMONIACAL NITROGEN BY AIR ASPIRATION AND SODIUM CARBONATE.

No. of sample.	Percentage of ammoniacal nitrogen with MgO. 0.5 gr.		Percentage of ammoniacal nitrogen. with Na_2CO_3.		Amount of Na_2CO_3 used.
No. 308		0.012		0.011	$^1/_2$ gram
No. 309		0.011		0.011	1 gram
	Duplicate	0.011	Duplicate	0.011	
No. 310		0.012		0.012	1 gram
	Duplicate	0.012	Duplicate	0.010	
No. 311		0.014		0.014	1 gram
	Duplicate	0.014	Duplicate	0.012	
No. 312		0.016		0.016	2 grams
	Duplicate	0.016	Duplicate	0.016	

A patient endeavor has been made in this laboratory to correlate the ammoniacal nitrogen evolved by the magnesium oxide distillation method with the condition of preservation of the chicken meat. While variations in the amount were visible when wide differences in the flesh were apparent to the sense of sight, odor, etc., small differences for which laboratory data were essential were more frequently confused than clarified by the analytical results.

Though the quantity of nitrogen obtained by air aspiration is always considerably less than is given off in the first hour by distillation, duplicate analyses check closely and individual samples having the same history show similar amounts of nitrogen with a gratifying regularity. Table IV illustrates this point and indicates, also, the variations by the distillation method when examining samples from the same source.

The results tabulated are divided into three groups—(*a*) chickens chilled in dry air at 0° for 24 hours after killing, which is sufficient to completely remove the animal heat; (*b*) chickens of known history preserved in a refrigerator having a temperature of 10–15° for varying numbers of days; (*c*) chickens, generally of known history, preserved hard frozen for varying periods of time.

It will be noted that the perfectly fresh birds show 0.011 to 0.012 per cent. of ammoniacal nitrogen in the tissue; those kept at moderately low temperatures for from 4 to 9 days run from 0.014 to 0.019 per cent.; while cold-stored specimens all more than a year old, show a wide variation in the quantities depending on their condition, but always much higher than the fresh. The ammoniacal nitrogen obtained from the aqueous extract of the tissue is generally a little less than from the tissue itself.

TABLE IV.—QUANTITY OF AMMONIACAL NITROGEN OBTAINED FROM CHICKEN FLESH OF VARYING QUALITY BY FOLIN METHOD.

	Description of sample.	Percentage ammoniacal nitrogen evolved.	Cu. ft. of air used.	Time consumed (in hours).	Percentage of ammoniacal nitrogen obtained by distilling 1 hour with MgO.
A	No. 255—Fowl 24 hours after slaughter, kept in chill room at 32° F. Dry picked.	0.012	10,500	5	.....
	No. 301—Fowl with same history as No. 255.	0.012	8,175	5	0.046
	No. 300—Same as 255.	0.011	4,375	4	.....
	No. 308—Same as 255.	0.012	6,550	4.5	0.041
	No. 309—Same as 255.	0.011	8,100	4.75	.....
B	No. 289–C—Fowl three days in house refrigerator.	0.014	8,302	6	.....
	Aqueous extract of No. 289–C.	0.012	8,302	6	.....
	No. 290—Fowl. Hard frozen. In transit four days.	0.014	8,900	5	0.036
	No. 291—Same history as No. 290.	0.014	8,900	5	0.030
	No. 311—Fowl 24 hours in chill room at 32° F. Six days in house refrigerator.	0.014	6,925	5	0.026
	No. 289–D—Same history as No. 311.	0.014	7,012	5	0.027
	No. 295—Fowl, five days in house refrigerator without previous chilling.	0.016	7,125	5	0.034
	No. 312—Fowl, 24 hours in chill room at 32° F. Seven days in house refrigerator.	0.016	8,080	6	.....
	No. 296—Fowl, 24 hours in chill room at 32° F. Nine days in house refrigerator.	0.016	14,850	6	.....
	No. 287—Fowl, 24 hours in chill room at 32° F. Six days in house refrigerator.	0.017	8,700	6.25	.....
	No. 288—Same history as 287.	0.018	8,700	6.25	.....
	No. 290–B—Fowl hard frozen. Four days in transit. Seven days in house refrigerator.	0.019	8,625	5	0.034
	No. 291–B—Same history as No. 290–B.	0.018	8,625	5	0.029
C	No. 302 — Chicken, cold-stored one year.	0.019	6,700	6	0.048

TABLE IV—(*Continued*).

	Description of sample.	Percentage ammoniacal nitrogen evolved.	Cu. ft. of air used.	Time consumed (in hours).	Percentage of ammoniacal nitrogen obtained by distilling 1 hour with MgO
C	No. 303—Same as No. 302.	0.019	6,700	6	0.030
	No. 253—Chicken of known history, two years in storage.	0.027	5,175	3.66	.....
	Aqueous extract of No. 253.	0.025	5,175	3.66	.....
	No. 254—Chicken of known history, two years in storage. Slightly gnawed by mice.	0.032	4,820	3.66	.....
	No. 256—Same as 254.	0.031	11,130	4.5	.....
	No. 298—Same as 254.	0.030	7,350	5	.....
	No. 258—Same as 254.	0.029	5,988	4.25	.....
	No. 299—Chicken of known history, two years in storage. Excellent condition.	0.023	11,610	8	0.036
	Aqueous extract 299.	0.022	11,610	8	.....
	No. 304—Chicken of unknown history in storage six years.	0.058	12,978	6	0.109

[CONTRIBUTION FROM THE FOOD RESEARCH LABORATORY, BUREAU OF CHEMISTRY, UNITED STATES DEPARTMENT OF AGRICULTURE.]

THE DETERMINATION OF THE ACID VALUE OF CRUDE FAT AND ITS APPLICATION IN THE DETECTION OF AGED FOODS.

BY M. E. PENNINGTON AND J. S. HEPBURN.

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During the study of the handling of poultry intended for food, at present under way in this laboratory, it has been found necessary to devise methods for a more accurate estimation of changes in the composition of flesh than have been in common use and which would be sufficiently prompt to permit of the testing of a larger number of samples each day than is ordinarily accomplished with a moderate laboratory force. The acidity of the fat of the chicken has been found to be a sensitive index of the state of preservation of the whole bird. It increases from a very low value in the freshly killed specimen to forty or fifty times the original number, according to the method of preservation and the length of the keeping period. However, the usual procedure for the estimation of the amount of free acid in the fat, that is, extraction with a suitable solvent, drying, etc., was so time-consuming that its use was greatly restricted. It seemed desirable, therefore, to so modify the method that accurate results could be obtained without such an expenditure of time.