from linseed oil where the absorption was more distinct. No reading was obtained with safflower, as on the addition of alcohol to the coloring matter, a white precipitate formed and the solution which remained after filtering was too weak to read. It is quite evident from this, its behavior towards bromine and its high reading in carbon bisulfide solution, that the coloring matter in this substance is not carotin. Rape seed and turnip gave high and low readings, respectively, which is not surprising in view of their behavior in the previous tests.

SUMMARY

From this work it would seem that carotin is contained in corn, squash, orange peel, flaxseed, mustard seed, and black sesame seed. Palmer and Eckles showed its presence in butter fat and beef tallow, Gill in palm oil, and it has long been known to be in carrots and grass. It does not seem to be present in rape seed, white sunflower, turnip, safflower, cottonseed, or turmeric.

In conclusion the writer wishes here to acknowledge his indebtedness to Messrs. James F. Maguire, Jr., and In-shing Wan, by whom the experimental work was performed.

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DETERMINATION OF LOOSELY BOUND NITROGEN AS AMMONIA IN EGGS¹

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The chemical methods for the detection of incipient decomposition in foods must be selected in accordance with the character of the substance under examination. As is well known, ammonia is one of the decomposition products of proteins, and the determination of loosely bound nitrogen as ammonia has proved to be one of the best chemical methods in general laboratory use for the grading of eggs.^{2,3}

The principle is that of Folin,⁴ namely, of aerating an alkaline fluid until all the loosely bound nitrogen is driven off as ammonia. This is caught in a known amount of standard acid for titration, or merely in an excess of acid for a colorimetric determination. The size of sample and the time in which it must be run are the determining factors in the selection of the method.

The apparatus used for this purpose has been changed from time to time as improvements were devised until it is now most satisfactory and may be of interest to those who have to deal with the determination of loosely bound nitrogen in biological material. Of the two optional methods of aeration (suction or blowing), the latter is preferable, for it is easier to keep the conditions of aeration constant, and this is

important in determining the length of time necessary for complete removal of the ammonia, as other experimenters have shown¹ and as has been our experience.

The titration method is preferred wherever possible, but in case a large number of samples are to be run in a short time, the colorimetric method can be substituted. In the latter case the amounts of ammonia to be determined are so small that great care must be exercised to keep the apparatus free from ammonium salts.

FIG. 1—APPARATUS FOR TITRATION METHOD

- A—Pipe from air pump.
 - B—Wash bottle containing 35 per cent sulfuric acid.
 - C—Pipe to which aeration cylinders are connected.
 - D—Aeration cylinder (14³/₄ in. high × 1¹/₂ in. inside diameter) containing sample. The glass tube for aeration extends to within 1/2 in. of the bottom and is open at the end.
 - E—Trap.
 - F—Flask in which ammonia is caught, containing 10 cc. of N/50 sulfuric acid plus 2 drops of 0.2 per cent methyl red (dissolved in alcohol), and 75 cc. of water.
 - G—Dispersion tube made according to method of Folin and Farmer [*J. Biol. Chem.*, 11 (1912), 493] to insure complete absorption.
- NOTE—It has been found by test that the ammonia is always completely absorbed in the one flask by this method.
- H—Water gauge for keeping air pressure constant and thus ensuring the passage of an equal volume of air through the cylinders in a given time.

DIRECTIONS FOR TITRATION METHOD

Mix samples well (preferably with one of the electric mixers in common use at soda fountains) and weigh out 25 g. Pour the bulk of the egg into the aeration cylinder D and transfer the remainder by means of four 25 cc. portions of distilled water, stirring each time with a rubber-tipped glass rod to remove the egg adhering to the sides of the weighing vessel. Add 75 cc. of alcohol, mix well, and let stand 15 min. Now add about one gram of sodium fluoride, 2 cc. of 50 per cent potassium carbonate and 1 cc. of kerosene. Connect the apparatus, blow air through until no more ammonia comes over, and titrate solu-

¹ Published by permission of the Secretary of Agriculture.

² M. E. Pennington and A. D. Greenlee, "An Application of the Folin Method to the Determination of the Ammoniacal Nitrogen in Meat," *J. Am. Chem. Soc.*, **32** (1911), 561.

³ H. W. Houghton and F. C. Weber, "Methods Adapted for the Determination of Decomposition in Eggs and in Other Protein Food Products," *Biochem. Bull.*, **1914**, 447.

⁴ *Z. phys. Chem.*, **37** (1902), 161.

⁵ P. A. Kober and S. S. Graves, "Quantitative Ammonia Distillation by Aeration, for Kjeldahl, Urea, and Other Nitrogen Estimations," *J. Am. Chem. Soc.*, **35** (1913), 1594.

tion in flask F with $N/50$ sodium hydroxide to ascertain how much of the 10 cc. of $N/50$ sulfuric acid, with which the experiment started, has been neutralized. The per cent of nitrogen is then calculated from $0.00028 \times \text{cc. of } N/50 \text{ H}_2\text{SO}_4 \text{ neutralized} \times 100 = \text{Per}$

25

cent Nitrogen.

Tests on standard ammonium sulfate solutions and on egg, at 25°C . (the usual temperature of the laboratory) and under 14 cm. water pressure, showed that the ammonia was all driven over in 4 hrs. If the pressure under which air is forced through is altered to any extent, it is necessary to alter the aeration time to correspond.

The air is passed through 35 per cent sulfuric acid in wash bottles as a precaution, although it has been found that the amount of ammonia present is not great enough to interfere. 35 per cent sulfuric acid are used, because at this strength the volume of the liquid is nearest constant under the conditions of humidity ordinarily prevailing in Philadelphia, and no attention is therefore required except to see that the acid is not neutralized. While, of course, the volume will increase or decrease somewhat, depending upon a high or low humidity, the change is not great.

By the use of a small concentration of potassium carbonate any perceptible hydrolysis of the egg protein is avoided.

At the end of 4 hrs.' aeration all loosely bound nitrogen originally present has been freed, and the quantity given off on aerating for 2 hrs. more is so small that it cannot be determined by titration. The potassium carbonate may be added in solid form, but for convenience it is preferable to add 2 cc. of a 50 per cent solution, using a 10 cc. graduated bacteriological pipette for measuring. Sodium carbonate may be used in place of potassium carbonate, but as it is much less soluble, it is not quite so convenient to use as the latter.

Eggs cannot be aerated satisfactorily without the addition of something to prevent foaming, and alcohol has been found to be most effective. Kerosene must be added also, as most of the alcohol evaporates after 2 hrs.' aeration. Kerosene carried over into the collecting flask does not interfere with the titration. The directions for adding 100 cc. of water to the egg before addition of the alcohol should be carefully followed; if the alcohol is added first, the egg is coagulated in a coarse, stringy condition, instead of finely divided, and is therefore not as efficiently aerated.

It has not been found advisable to correct for a blank. The following table shows the reason, namely, that in many cases no blank at all is obtained; where there is one, it is too small to be of practical importance.

TABLE I—12 BLANKS COMPOSED OF 1 G. Na_2CO_3 , 1 G. Na_2F_2 , 75 CC. ALCOHOL AND 125 CC. OF WATER. AERATED $1\frac{1}{2}$ HRS. THROUGH 5 CC. $N/50$ ACID AND THEN TITRATED AGAINST $N/50$ NaOH

$N/50$ NaOH used—cc.	4.95	5.00	5.04	4.96	4.95	5.02
Blank—cc.	0.05	0.00	0.04	0.04	0.05	0.02+
$N/50$ NaOH used—cc.	4.95	4.95	4.95	5.00	4.90	4.95
Blank—cc.	0.05	0.05	0.05	0.00	0.10	0.05

Sodium hydroxide cannot be used as the alkaline agent because continuous hydrolysis takes place

with the liberation of ammonia, and no definite stopping place is reached. This holds even in the presence of sodium chloride to decrease the ionization, and is well shown in the following table.

TABLE II—COMPARISON OF NaOH AND Na_2CO_3 IN THE PRESENCE OF Na_2F_2 AND NaCl. SAMPLE RUN IN TRIPPLICATE

1 G. Na_2CO_3 and 1 G. Na_2F_2	0.8 G. NaOH and 1 G. Na_2F_2	1 G. NaOH and 15 G. NaCl	3 G. NaOH and 15 G. NaCl
Per cent N (a)	Per cent N	Per cent N	Per cent N
0.0032	0.0059	0.0054	0.0104
0.0029	0.0052	0.0056	0.0112
0.0032	0.0041	0.0050	0.0112

(a) It should be mentioned at this point that all percentages given in this paper are on the wet basis; that is, on the weight of egg fresh from the shell, or in the case of frozen egg, immediately after thawing.

The action of magnesium oxide as compared with potassium carbonate is shown in the following table. The sample was run as usual, in triplicate for each, the only variation being in the substitution of magnesium oxide for potassium carbonate.

TABLE III

Run as Usual 1 g. K_2CO_3 Per cent N	Run as Usual 1 g. MgO Per cent N	Repeated after K_2CO_3 Sample Per cent N	Repeated after MgO Sample Per cent N
0.0031	0.0030	0.0007	0.0018
0.0030	0.0031	0.0005	0.0014
0.0030	0.0032	0.0004	0.0014

It is seen that the differences on the first run are within the limit of error, but after having allowed both portions to stand over night ($18\frac{1}{2}$ hrs.), then adding 75 cc. alcohol and 25 cc. water, and again aerating 4 hrs., more ammonia was given off by the magnesium oxide sample, indicating that in this particular case the magnesium oxide is more active than the potassium carbonate.

Table IV shows the result of using sodium hydroxide and sodium carbonate in place of potassium carbonate. One gram of each was used and the sample run in triplicate, otherwise as usual.

TABLE IV

K_2CO_3 Per cent N	Na_2CO_3 Per cent N	NaOH Per cent N
0.0029	0.0030	0.0044
0.0029	0.0029	0.0048
0.0029	0.0029	0.0044

It will be noted that potassium carbonate and sodium carbonate give the same results, whereas the results obtained by the use of sodium hydroxide are much higher. There is no definite end-point with sodium hydroxide; ammonia is given off continuously for a long time.

To illustrate the practical application of this method, the following tables and explanations are given.

Table V includes results obtained on whites, yolks, and whole eggs, fresh, and after 10 mos. in cold storage. They were so-called "April Firsts," the best grade for storage. The figures show clearly that no change takes place as regards the loosely bound nitrogen of the white, the increase for the amount in whole egg being referable solely to the rise of loosely bound nitrogen in the yolk. It also shows how close are the results on individual eggs of the same grade computed on the moist basis.¹

¹ It might be stated here that determination of ammonia in dried egg is of little value, for it has been shown that ammonia is driven off during commercial drying. See U. S. Dept. of Agriculture, *Bulletin* 51, "A Bacteriological and Chemical Study of Commercial Eggs in the Producing District of the Central West," M. E. Pennington, et al.

TABLE V—INDIVIDUAL EGGS—"APRIL FIRSTS"

FRESH			AFTER 10 MONTHS IN COLD STORAGE		
White Per cent N	Yolk Per cent N	Whole Egg Per cent N	White Per cent N	Yolk Per cent N	Whole Egg Per cent N
0.0005	0.0029	0.0013	0.0003	0.0060	0.0025
0.0004	0.0031	0.0013	0.0004	0.0056	0.0026
0.0003	0.0028	0.0011	0.0003	0.0062	0.0022
0.0004	0.0028	0.0012	0.0004	0.0065	0.0035
0.0005	0.0031	0.0014	0.0005	0.0059	0.0031
0.0003	0.0034	0.0013	0.0004	0.0062	0.0030

Table VI shows results on liquid egg held frozen for some months. No apparent increase of loosely bound nitrogen is indicated in edible eggs held hard-frozen for 9 mos. If the eggs are held in the shell for that length of time at slightly above freezing, a gradual increase does take place, however. This is well shown in Table VII.

TABLE VI—LIQUID EGG HELD FROZEN		
When Put in Freezer Per cent N	Months Held Frozen	After Coming from Freezer Per cent N
0.0026	3	0.0025
0.0030	3	0.0031
0.0024	9	0.0026
0.0022	9	0.0024
0.0025	9	0.0025

TABLE VII—CASE EGGS HELD IN STORAGE—"JUNE FIRSTS"
(Eggs in each sample—2 dozen—about 1 kg.)

Months	Per cent N
0	0.0013
1	0.0011
2	0.0022
3	0.0021
4	0.0028
5	0.0032
6	0.0032
7	0.0030
8	0.0035
9	0.0040

MICROCHEMICAL METHOD

The principle of this method is the same as for the titration method, but in operating, scrupulous cleanliness is compulsory for even minute amounts of ammonium salts in apparatus or reagents will, of course, invalidate the results.

Five cubic centimeters of liquid egg are placed in a 250 cc. Erlenmeyer flask and 45 cc. of phosphotungstic acid solution (made up in the ratio of 10 cc. of 1 per cent sulfuric acid, 20 cc. of 20 per cent phosphotungstic acid, and 420 cc. of water) added. Shake well, and let stand 5 min.; then filter through an ammonia-free folded filter.¹

Ten cubic centimeters of the phosphotungstic acid filtrate are transferred to the large test tube D, shown in Fig. II; 1 cc. of 10 per cent sodium hydroxide and 2 drops of heavy white paraffin oil are added, then the solution is aerated as fast as possible into 2.5 cc. of 1 per cent sulfuric acid for one-half hour.

The dispersion tube is rinsed into the collector with ammonia-free water, 1 cc. of Nessler-Winkler solution added, and the volume then made up to 10 cc. with water. Comparison is made with pure standard ammonium sulfate solution in a Duboscq or Schreiner colorimeter.

Directions for making pure ammonium sulfate may be found in the article by Folin and Farmer previously referred to, and for Nessler-Winkler solution in *Chem. Zentr.*, 11 (1899), 320, or "Analytical

¹ Test filter papers by soaking a packet of them in ammonia-free water. If a portion of this water shows much ammonia when nesslerized, the water is poured off and allowed to drain from the filter papers. The soaking and testing are repeated until the ammonia is removed; the papers are then dried.

Chemistry," Treadwell-Hall, I, 46. Ammonia-free water is prepared for use by the method of J. Barnes,¹ in which the water is boiled with enough bromine to color it. Before using, test with starch-potassium iodide solution to be sure all bromine has boiled off.

A comparison of results by the colorimetric and titration methods is given in Table VIII. They show that with very minute amounts of ammonia the colorimetric figures are a little high, but when dealing with amounts of ammonia (expressed as nitrogen) ranging from 0.0020 to 0.0040 per cent (0.02 to 0.04 mg.) the agreement is good. If possi-

FIG. II—APPARATUS FOR MICROCHEMICAL METHOD

- A—Acid wash bottles.
B—Air pipe leading from A, to which aeration tubes are attached.
C—Dispersion tube reaching to within 1/4 in. of the bottom of the large test tube D.
D—Large test tube for holding sample—10 in. high by 1 1/8 in. inside diameter.
E—Trap.
F—Dispersion tube for complete absorption. Tubes C and F are made according to the directions of Folin and Farmer referred to in the preceding method.
G—Special form of test-tube for catching ammonia without splashing liquid out of the tube. Its dimensions are—height, 6 1/2 in., inside diameter 5/8 in., diameter of bulb 1 3/4 in. A mark is placed on the constriction at the 10 cc. point.
A 1/4 h. p. motor is used to drive the air pump.

ble, it is well to use aliquots of the egg filtrate which will bring the amount of ammonia within these limits. When dealing with bad eggs there is often so much ammonia present that a heavy brick-red precipitate forms on adding the Nessler solution. This may be overcome by adding just enough 5 per cent acetic acid to dissolve it, diluting to about 45 cc., again nesslerizing and making up to 50 cc. Of course it is necessary to make a corresponding change in the calculation.

TABLE VIII—COMPARISON OF RESULTS BY COLOR AND TITRATION

White		Yolk		Whole Egg	
Per cent N by Color	Per cent N by Titration	Per cent N by Color	Per cent N by Titration	Per cent N by Color	Per cent N by Titration
0.0010	0.0005	0.0033	0.0029	0.0018	0.0013
0.0009	0.0004	0.0030	0.0031	0.0016	0.0013
0.0010	0.0003	0.0030	0.0028	0.0017	0.0011
0.0009	0.0004	0.0031	0.0031	0.0016	0.0012
0.0010	0.0005	0.0028	0.0034	0.0016	0.0014

Table IX shows the results obtained when sodium hydroxide, potassium carbonate and a mixture of potassium carbonate and potassium oxalate are used in the colorimetric method as reagents for making alkaline before aeration. It will be seen that there is no difference which is not within the limit of experimental error.

¹ *J. Soc. Chem. Ind.*, 15 (1896), 254.

TABLE IX—COMPARISON OF K_2CO_3 , $NaOH$ AND A MIXTURE OF K_2CO_3 AND $K_2C_2O_4$ IN THE COLORIMETRIC METHOD

SAMPLE No.	1 Cc. of 10 Per cent $NaOH$	4 Drops of (8 Per cent K_2CO_3 + 12 Per cent $K_2C_2O_4$ in Equal Parts)		3 Drops of 5 Per cent K_2CO_3
		K_2CO_3	$K_2C_2O_4$	
1.....	0.0024	0.0022	0.0025	0.0025
2.....	0.0023	0.0025	0.0021	0.0021
3.....	0.0021	0.0026	0.0018	0.0018
4.....	0.0027	0.0025	0.0025	0.0025
5.....	0.0026	0.0025	0.0027	0.0027
6.....	0.0026	0.0026	0.0026	0.0026

SUMMARY

I—Titration and colorimetric methods for the determination of the small amounts of loosely bound nitrogen in liquid eggs by aerating the alkaline material (according to the principle of Folin) have been presented, with descriptions of the apparatus and precautions necessary.

II—The effect of various agents used to make the material alkaline, some results on different grades of eggs, and a comparison of results obtained by both methods are shown in the tables.

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A METHOD FOR THE DETECTION OF FOREIGN FATS IN BUTTER FAT

By ARMIN SEIDENBERG
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The methods at present available for the detection of foreign fats in butter fat are such that in many cases considerable difficulty is experienced in definitely ascertaining the addition of limited quantities. The constant which has been found to be of most significance in the analysis of butter fat is the Reichert-Meissl number. This constant is dependent upon the soluble volatile acids which are particularly characteristic of butter fat. While the other constants which are usually depended upon in determining the purity of fats and oils, such as the specific gravity, refractive index, melting point, saponification number, iodine number, etc., have in the case of butter fat well-defined limits, sufficiently distinct to permit the identification of an unadulterated butter fat, they nevertheless in many instances lie so close to those of other fats that considerable quantities of these can be added to a butter fat without thereby transgressing the limits for a known pure sample.

THE REICHERT-MEISSEL NUMBER

Among the more commonly known edible fats and oils none has a Reichert-Meissl number that approaches the minimum and maximum limits of this constant for butter fat. These range according to the numerous authorities cited by Lewkowitsch¹* from 17 to 36.3. According to Sherman² the usual variations in the Reichert-Meissl value for butter fat are between 24 and 34; cocoanut oil, with a Reichert-Meissl number of 6 to 8, has the next highest value among the more widely used edible fats and oils, while the other edible fats and oils usually have numbers below 1. According to Lewkowitsch³ the lowest Reichert-Meissl value adopted by analysts in various countries, although not officially, lies between 23 and 25. He

* Numbers refer to corresponding numbers in "References," p. 621.

states that values need not fall below this in a legitimate sample of butter fat if proper precautions in feeding, etc., are taken. In view of the fact, however, that a large number of undoubtedly pure samples of butter fat have been observed with Reichert-Meissl values below these, it is evident that no butter can be adjudged legally adulterated unless its constants are all decidedly below the lowest observed limits of a pure sample.

The wide variation of the Reichert-Meissl number makes it feasible to add considerable quantities of foreign fats to a butter with a high value without thereby causing it to fall below the lower limit. The problem is further complicated according to Lewkowitsch⁴ by the fact that glycerides or ethers of the volatile fatty acids such as tributyrin or amyl acetate may be added to an adulterated butter fat in order to raise its Reichert-Meissl value. He states that even without counteracting the effects of sophistication by this means it is not possible to detect in every case the presence of 10 or even 20 per cent of a foreign fat in butter fat by means of the Reichert-Meissl value. Indeed, it is quite evident that on a butter having a Reichert-Meissl value of 33 or above, adulteration to the extent of 25 and 30 per cent may be practiced without lowering the value below the lowest observed limits. Thus the writer found on adding 30 per cent of tallow to a butter fat having a Reichert-Meissl number of 33.9 that the value was decreased to only 24.7. The refractive index of the sample containing the 30 per cent added tallow was 1.4588, which is still within the limits that have been observed for a pure sample. None of the other constants usually determined would be affected to a greater extent. Methods depending upon determining the amount of stearic acid present are likely to prove uncertain, particularly in view of the varying amounts of this acid found in butter fat by different observers.

COMPOSITION OF BUTTER FAT

The constants of any fat or oil are of course dependent upon the glycerides which it contains and upon the fatty acids which constitute these glycerides. The fatty acids generally considered to be present in butter fat are butyric, caproic, caprylic, capric, lauric, myristic, palmitic, and stearic. The comparatively large amount of the lower soluble volatile fatty acids are peculiarly characteristic of butter fat and it is upon these that the Reichert-Meissl number depends. Their amount in proportion to the other fatty acids is, however, not very large. According to Browne the total amount of butyric and caproic acids present in butter fat is 7.54 per cent. Duclaux⁶ found butter fat to contain 2 to 2.26 per cent caproic acid and 3.37 to 3.65 per cent butyric acid. Browne states the amount of oleic acid to be 32.50 per cent, of palmitic acid 38.61 per cent, and of stearic acid 1.83 per cent. According to a large number of butters of varied composition examined by Siegfeld⁷ the volatile soluble acids range from 5.60 to 7.09 per cent, the volatile insoluble acids from 0.95 to 3.28 per cent, the saturated nonvolatile acids from 40.65