# Colorimetric Method for Lipoid Phosphoric Acid in Eggs and Noodles

JOHN E. DESPAUL, ALBERT WEINSTOCK[1], and CHARLES H. COLEMAN

Quartermaster Subsistence Testing Laboratory, Chicago Quartermaster Inspection Service Field Office, Chicago 9, Ill.

The use of lipoid phosphoric acid as the conventional index of the egg content of noodles has been criticized because lipoid phosphoric acid deteriorates during prolonged storage and it has been difficult to obtain reproducible results. The authors show that phospholipides in frozen eggs will remain stable for 36 months in freezer storage without appreciable deterioration due to the presence of microorganisms; and phospholipides in egg noodles will remain stable for 6 months at room temperature. Therefore, lipoid phosphoric acid is a reliable quantitative index for egg products which are fresh or have not been in storage in excess of 6 months. Although poor reproducibility can be overcome by competent chemists exercising the care and skill demanded by the official procedure, this does not hold true for the less expert technician. The method given facilitates performance of the determination and provides better agreement in the results obtained by the average technician. The method reduces potential sources of quantitative error by the use of a wet-ash colorimetric method for determining lipoid phosphoric acid. It eliminates several tedious quantitative transfers and employs an instrumental method of measurement. Data obtained by a number of technicians show improved reproducibility of results over those obtained with the dry-ash volumetric method. The substantial saving in time and the greater reliability of results are of particular significance to laboratories engaged in the quality control of egg products.

A RELIABLE AND RAPID METHOD, for ultimate use in determining compliance with contract requirements for the egg content of noodles, has been sought by this laboratory. Three fundamental methods are available for determining the egg content of alimentary pastes, but each has certain limitations. In the first, cholesterol is determined and the egg content computed by the procedure developed by Haenni (*5*). Because of the stability of cholesterol, Haenni's method is a reliable index, and is highly recommended by investigators, especially when long-term storage studies are involved. Tillmans, Riffart, and Kühn (*18*) have shown that the cholesterol content of eggs and egg products remains relatively constant during storage. Haenni kept samples of ground noodles in sealed jars in the laboratory for 4 years, during which time they had developed a strong musty odor. Upon analysis, essentially all of the cholesterol originally present in the samples was recovered. However, the cholesterol method is not practical for routine determinations of egg content, as it requires the patient application and industry of an able and experienced chemist to obtain accurate and reproducible results. Consequently, Haenni's method is little used for determining the egg content of noodles.

A different approach, proposed by Munsey (*13*), relies upon the determination of choline as an index of the egg content. Collaborative studies, in which this laboratory participated, were conducted by Munsey in 1952, using the new method—the first time that the choline procedure had been studied collaboratively as a practicable means of determining egg content. Although some of the analysts showed good agreement, Munsey felt that changes were warranted. Consequently, it was recommended that the method for choline in noodles be given further study (*8*).

The third method—and the one on which this laboratory has concentrated—utilizes lipoid phosphoric acid as the determinant, and follows the procedure proposed by Hertwig (*6*). Not only has the lipoid phosphoric acid method had official status with the Association of Official Agricultural Chemists since 1926, but the literature also reveals extensive research using lipoid phosphoric acid as an index of egg solids. Despite these two sanctions, the method has been subjected to considerable criticism by investigators. Their reluctance to accept the method as it is currently used is based upon two important considerations: deterioration of the lipoid phosphoric acid in eggs and noodles during storage, and lack of good agreement in lipoid phosphoric acid determinations among different analysts working on the same sample.

### Deterioration in Storage

Hertwig, and Buchanan (*3*), both found that the recovery of lipoid phosphoric acid was approximately 90% in noodle samples of known composition. Hertwig had suggested that a factor of 1.1 be used to compensate for this 10% loss of lipoid phosphoric acid, which apparently occurred during the manufacturing process. Consequently, this factor was incorporated in the Hertwig formula as reported by Buchanan, and has been used through the years.

While freshly prepared noodles yield 90% lipoid phosphoric acid, investigators of long-term storage effects concur in the belief that phospholipides deteriorate, and therefore lipoid phosphoric acid loses its value as a reliable index of egg solids. Hertwig recovered only 81% lipoid phosphoric acid from noodles stored for 1 year. Buchanan, storing

[1] Present address, Armour Research Foundation, Chicago 16, Ill.

samples of ground noodles for a year, obtained a yield of only 70% egg solids by the lipoid phosphoric acid method. It is evident that a partial breakdown of phospholipides into inorganic phosphorus occurs when noodles are subjected to prolonged storage, or storage under adverse conditions. When analyzed by Hertwig's method, this inorganic phosphorus is not recovered. On the other hand, egg noodles stored at room temperature for periods up to 6 months do not show an appreciable decrease in lipoid phosphoric acid. This is well illustrated by the work of Koehn and Collatz (*9*), who compared the lipoid phosphoric acid and cholesterol methods in a study of the egg content of noodles stored for 5 to 6 months. About 98% of the egg solids was recovered in these samples by the lipoid phosphoric acid method applying the 1.1 factor, as compared with the cholesterol method. It would appear that deterioration is accelerated during the second 6-month storage. Mitchell's (*11*) work is also significant in that the lipoid phosphoric acid content of eggs was not materially affected by the storage of thawed frozen eggs at room temperature for 6 days, after which time the odor was putrid.

The effect of storage must always be considered in terms of the objective which the test is intended to attain. Measuring the egg solids content of samples, representing a long-term storage product, is one objective; testing currently manufactured noodles is another. The Quartermaster Subsistence Testing Laboratory, which is concerned with the acceptance testing of egg noodles currently being manufactured, is interested in the second objective. The time interval between actual manufacture and laboratory analysis is such that, barring the 10% loss during the manufacturing process, the phospholipides do not deteriorate to a significant extent. Therefore, lipoid phosphoric acid can be relied upon as being indicative of the egg solids content. Results obtained by this laboratory, and by the aforementioned investigators, indicate that if fresh eggs, or fresh eggs that have been frozen or dried, are used in preparing egg noodles, the lipoid phosphoric acid is a reliable index of the egg solids content of the noodles, if the product is analyzed soon after being manufactured. These conditions are met in samples of noodles that are received in this laboratory for determination of egg content. Because the time element between the manufacture and testing of noodles for military procurement is short, the instability of phospholipides during storage periods has no bearing on the use of the method for acceptance testing of noodles in this laboratory. The lipoid phosphoric acid method is to be preferred for acceptance testing not only because it is more readily applied than the cholesterol method, but because it also would tend to disqualify noodles tendered from old stock.

**Table I. Lipoid Phosphoric Acid in Frozen Egg Yolk**

| Storage Time, Months (at −20° F.) | Lipoid Phosphoric Acid in Frozen Egg Yolk (Dry Basis), % | | | |
|---|---|---|---|---|
| | Official Method | | Proposed Method | |
| | Sample A | Sample B | Sample A | Sample B |
| 1 | 1.89<br>1.92 | ... | ... | ... |
| 2 | 1.91<br>1.90<br>2.10<br>2.10<br>2.09 | ... | 2.06[a]<br>2.06[a] | ... |
| 3 | 1.93<br>1.94<br>2.09<br>... | ... | 2.04[b]<br>2.04[b]<br>2.04[a]<br>2.04[a] | ... |
| 4 | 2.00<br>1.99 | ... | 1.98[a]<br>1.98[a] | ... |
| 17 | ... | 1.90<br>1.90 | ... | ... |
| 18 | ... | 1.96<br>2.18<br>2.18<br>2.18 | ... | 2.04[a]<br>2.04[a] |
| 19 | ... | 1.88<br>1.88<br>2.03 | ... | 2.07[a]<br>2.07[a]<br>1.99[b]<br>1.99[b] |
| 20 | 1.87<br>1.87 | 1.92<br>1.96 | 1.85[c]<br>1.87[c] | 1.96[a]<br>1.97[a] |
| 36 | ... | 1.87<br>1.86 | ... | 1.85[c]<br>1.96[c] |

[a] Wet-ash volumetric.
[b] Dry-ash colorimetric.
[c] Wet-ash colorimetric.

In the past 4 years, some 1500 determinations of lipoid phosphoric acid in 357 samples of egg noodles have been performed in this laboratory to determine compliance with contract requirements of the egg content. A compilation of the results obtained shows that approximately 90% of the samples submitted readily met contract requirements. Five per cent failed initially, but passed on subsequent samples submitted for reinspection. The remaining 5% were decidedly below the specification minimum.

### Reproducibility of Results

Collaborative studies show that the official lipoid phosphoric acid method for determining the egg solids content of noodles is subject to a wide range of results obtained by analysts working on the same sample in various laboratories. For a sample of noodles made up with 5.5% egg yolk solids, Munsey (*12*) found that 10 collaborators obtained results varying from 3.5 to 5.6%. Similar results, varying from 4.3 to 6.5% were obtained on a sample containing 5.5% whole egg solids. The analysts followed a slight modification of the conventional procedure. A subsequent study of the lipoid phosphoric acid method, on both egg yolk and whole egg noodles, conducted by Munsey in 1952, showed a comparable wide range in results obtained by 9 collaborators. For a sample of noodles containing 5.7% egg yolk solids, the results ranged from 5.3 to 6.6% while the results for a sample of 5.3% whole egg noodles varied from 3.3 to 6.0%. As a result of this study the Association of Official Agricultural Chemists deleted the official method for lipoid $P_2O_5$ from its compendium (*2*) and redesignated it as "first action," incorporating the slight modification used collaboratively (*7*).

Observations in this laboratory indicate that lack of agreement in results is due to faulty performance of the test. Difficulty in obtaining check results may be caused by the several quantitative transfers and operations involved in the analysis, with frequent chance for error or loss. Losses from the platinum dishes may be due to spattering during evaporation of chloroform on the steam bath. To obviate this source of error this laboratory has used a current of air for the chloroform evaporation, followed by careful drying in an oven. The dry residue is then charred in a muffle furnace at 500° C. The residue must be carefully charred at a lower temperature prior to placement in the 500° furnace; otherwise, sudden burning will occur with consequent loss of phosphorus-containing particles. If the residue is not well charred, complete recovery of phosphorus cannot be expected. After

charring, the ash is dissolved in dilute nitric acid. During the addition of the acid considerable spattering occurs; therefore great care must be taken to prevent loss at this point. The subsequent transfer, filtration, and titration steps are also conducive to error. High values are caused by incomplete washing of the ammonium phosphomolybdate that is formed, or by use of distilled water with a low pH.

Winston (*19*) states that in order to ensure complete extraction of the phosphorus from the charred residue, it is necessary to repeat the ashing of the residue on the filter paper and combine the filtrates from the double ashing procedure. Variations in the official procedure for determining the lipoid phosphoric acid may affect the final result sought when an empirical formula is applied to arrive at the egg solids content. If such a formula was initially derived from data obtained by a prescribed method, then an improved method, giving a greater recovery of phosphorus, would require a formula having revised constants in order to give a result indicative of the egg solids content. The trend in current official compendia and government specifications is to use analytical determinants directly as an index of food quality. This eliminates the use of formulas and conversion factors, thus avoiding any differences in opinion that might arise with regard to the applicability of such formulas. Present government specifications express the requirements for egg solids in noodles in terms of lipoid phosphoric acid without applying the 1.1 recovery factor for loss during the manufacturing process, and without using any formula to convert to per cent egg solids. Therefore, the data given herein for egg solids in noodles are reported as per cent lipoid phosphoric acid rather than as per cent egg solids.

If the painstaking care that the analysis requires is exercised, reliable results are readily obtainable by the official procedure, even though it be tedious and time-consuming. In a number of instances where this laboratory's findings of deficiency in egg content were challenged, reproducible results were obtained on the disputed noodle samples by the contractors' consulting chemists and the authors. The agreement in such cases was usually within 0.1% egg solids content. Lack of good agreement is not, therefore, due to any inherent fault with the lipoid phosphoric acid method, but, rather, to faulty technique in the application of the procedure.

The proposed wet-ash colorimetric method was developed by the authors from the reaction of Fiske and Subbarow (*4*), as modified by Snell (*16*), in order to circumvent the above-noted sources of error.

### Procedure

**Reagents.** 1-Amino-2-naphthol-4-sulfonic acid solution. 0.25%. Dissolve 1.0 gram of ANSA reagent in 390 ml. of 15% sodium pyrosulfite solution. Add 10 ml. of 20% sodium sulfite solution to clear. Filter. Prepare fresh every 2 weeks.

Ammonium molybdate solution, 2.5%. Dissolve 13.9 grams of ammonium molybdate in 500 ml. of 5 *N* sulfuric acid.

Standard phosphorus solution. Dissolve 0.4394 grams of potassium dihydrogen phosphate in water, add 5 ml. of 1 *N* sulfuric acid, and dilute to 1 liter. Dilute 25 ml. of this solution to 250 ml. Each milliliter contains 0.01 mg. of phosphorus. For accurate work the phosphorus content of the reagent should be determined analytically.

**Extraction.** Transfer 5 grams of ground noodles into a 250-ml. centrifuge bottle and add 15 ml. of 70% alcohol, swirling to moisten all particles. Cover loosely with a cork stopper and set in a water bath at 75° to 80° C. After the contents reach this temperature, continue heating for 15 minutes, swirling every few minutes. Add 27 ml. of 95% alcohol, cover tightly with a rubber stopper, and shake for 2 minutes. Cool, add 45 ml. of ether, and shake for 5 minutes. Centrifuge, and decant liquid through glass wool into a 250-ml. beaker. Rinse the bottle neck with ether. Repeat extraction of the residue with three successive 20-ml. portions of ether, breaking up the residue with a glass rod, shaking 2 minutes, and centrifuging each time. Evaporate the solvent from the combined extract in the draft of a fume hood. Remove the remaining moisture by drying in a 100° C. oven for 10 minutes. Dissolve the lipoids in about 15 ml. of chloroform. Filter through glass wool into a 250-ml. Erlenmeyer flask. Complete the transfer of the lipoids with the aid of a stream of chloroform from a wash bottle. Evaporate the solvent on a steam bath.

**Digestion.** To the residue add 10 ml. of nitric acid, swirl, and let stand for 5 minutes. Add 5 ml. of sulfuric acid and heat on a hot plate until sulfur trioxide fumes appear. Cool, add about 1 gram of sodium nitrate, and heat until sulfur trioxide fumes appear. Repeat the addition of sodium nitrate and heating to sulfur trioxide until the digest is clear and colorless. Cool, add 50 ml. of water, and again boil until sulfur trioxide fumes appear. Repeat the addition of water and heating to sulfur trioxide fumes to assure removal of nitrogen oxides. Cool, dilute with 50 ml. of water, and neutralize with 50% sodium hydroxide, using 1 drop of phenolphthalein. Make acidic with a drop of sulfuric acid. Transfer to a 250-ml. volumetric flask for lipoid phosphorus pentoxide in the range of 5.5% egg noodles. This is the sample solution.

**Colorimetry.** Pipet 25 ml. of sample and standard solutions into two 100-ml. volumetric flasks. Dilute each to about 50 ml. with water. Add 20 ml. of the 2.5% ammonium molybdate reagent, swirl, and let stand at least 15 minutes. Add 10 ml. of 1-amino-2-naphthol-4-sulfonic acid reagent and immediately dilute to the mark with water. Mix by inverting four times. Place in a 37° C. water bath for 30 minutes, and mix at 10-minute intervals. At the end of the 30-minute period transfer to matched cells for reading in a photoelectric colorimeter. Adjust the instrument to show 37% transmittancy at 650 mμ for the standard, and obtain the comparative reading for the sample. The transmittancy for the sample should be within a few percentage units of the standard. If it is not, take a sample aliquot giving a reading near the standard. This procedure is preferable to the preparation of a standard curve, owing to slight changes in the curve from day to day. The analyses should be completed promptly, for the prepared sample solution will support the growth of microorganisms with consequent low recovery of phosphorus.

**Table II. Lipoid Phosphoric Acid in Frozen Egg Yolk**

| | *Lipoid Phosphoric Acid in Frozen Egg Yolk (Dry Basis), %* | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Method* | *1* | *2* | *3* | *4* | *5* | *6* | *7* | *8* | *9* | *10* | *11* |
| Dry ash | 1.96 | 1.93 | 1.94 | 2.03 | 1.92 | 1.96 | 2.09 | 2.00 | 1.98 | ... | ... |
| Wet ash | 1.96 | 2.00 | 2.01 | 2.03 | 2.02 | 2.02 | 1.99 | 1.96 | 1.97 | 1.98 | 1.98 |

**Table III. Lipoid Phosphoric Acid in Frozen Egg Yolk**

| | *Sample A* | | | | *Sample B* | | |
|---|---|---|---|---|---|---|---|
| *Colorimetric Method, Ml.* | *30* | *40* | *50* | *60* | *30* | *40* | *50* |
| Micrograms P per ml. | 5.03 | 6.65 | 8.38 | 9.99 | 4.97 | 6.60 | 8.25 |
| Optical density | 0.624 | 0.824 | 1.038 | 1.222 | 0.615 | 0.817 | 1.022 |
| % lipoid $P_2O_5$ in frozen egg yolk, D.B. | 2.00 | 1.98 | 1.99 | 1.98 | 1.98 | 1.97 | 1.97 |

Average of 25 official determinations = 1.98%

## Table IV. Lipoid Phosphoric Acid in Egg Noodles

| | Lipoid Phosphoric Acid in Egg Noodles[a] (Dry Basis), % | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Sample | Official | Wet-Ash Volumetric | Sample | Official | Wet-Ash Volumetric | Sample | Official | Wet-Ash Colorimetric |
| A | 0.138 | 0.136 | I | 0.147 | 0.145 | M | 0.130 | 0.130 |
| | 0.136 | 0.139 | | 0.147 | 0.147 | | 0.132 | 0.130 |
| | 0.138 | 0.139 | | 0.149 | | | 0.125 | 0.132 |
| | 0.138 | 0.138 | | 0.144 | | | 0.126 | 0.132 |
| | 0.139 | 0.138 | | 0.145 | | | 0.123 | 0.126 |
| B | 0.126 | 0.126 | | | | | 0.132 | 0.132 |
| C | 0.117 | 0.117 | | | | | 0.132 | 0.132 |
| D | 0.149 | 0.147 | | | | | 0.132 | 0.130 |
| | 0.147 | ... | | *High Content* | | | 0.130 | 0.130 |
| E | 0.108 | 0.110 | | *Whole Egg Noodles* | | | 0.133 | 0.130 |
| | ... | 0.111 | | | | | 0.133 | 0.130 |
| F | 0.138 | 0.139 | | | | | 0.126 | 0.126 |
| | ... | 0.139 | | | | N | 0.110 | 0.110 |
| G | 0.132 | 0.132 | J | 0.166 | 0.166 | | 0.110 | 0.110 |
| | 0.132 | 0.132 | K | 0.222 | 0.222 | O | 0.138 | 0.140 |
| | ... | 0.132 | | 0.220 | 0.222 | | 0.136 | 0.140 |
| H | 0.130 | 0.133 | L | 0.215 | 0.215 | P | 0.135 | 0.137 |
| | ... | 0.132 | | 0.215 | 0.215 | | 0.136 | 0.137 |

[a] Recovery factor of 1.1, to account for loss of lipoid phosphoric acid during manufacturing process not applied.

### Calculation of Results

$$\text{Per cent lipoid } P_2O_5 \text{ (dry basis)} = \frac{0.462 \times 0.573 \times 250 \times 100}{0.432 \times 5 \times 25 \times 0.900 \times 1000} = 0.136$$

where

0.462 and 0.432 are the optical densities of the sample and standard, respectively.

0.573 is the quantity of $P_2O_5$ in milligrams represented by 25 ml. of the standard.

250 is the dilution volume of the sample in milliliters.

100 is the factor for converting grams to per cent.

5 is the sample weight in grams.

25 is the volume in milliliters of sample aliquot taken.

0.900 is the fraction of dry substance in the sample.

1000 is the factor for converting milligrams to grams.

### *Results and Discussion*

This method eliminates a number of steps, including evaporation of solvent from shallow platinum dishes, ashing operations, and several quantitative transfers. It also makes use of an instrumental method for measurement, thereby precluding the need for observation of a difficult end point in titration. The new method saves time, reduces personal error, and limits the demand in the degree of manipulative skill required. The phospholipides are extracted in the conventional manner and transferred to an Erlenmeyer flask, where the solvent-free residue is digested with sulfuric acid and nitrate. Phosphorus is determined in an aliquot of the digest by developing the molybdenum blue color, using 1-amino-2-naphthol-4-sulfonic acid as a reducing agent. The transmittancy is measured in a photoelectric colorimeter at 650 mμ and compared with the standard phosphorus solution.

In recent years, the greater proportion of egg noodles that have been manufactured for military procurement have been produced using commercial frozen egg yolk for the egg component. The Quartermaster Subsistence Testing Laboratory was interested to note whether the time of storage of the frozen egg yolk component used had any effect on the available lipoid phosphoric acid from the phospholipides of this yolk. Consequently, the stability of the phospholipides in frozen egg yolk was studied during the past 3 years, at the same time that a comparison study of the lipoid phosphoric acid methods was made. The results of this storage study are shown in Table I. Both samples of egg yolk were in 30-pound commercial cans prepared at Swift and Co.'s Sioux City, Iowa, plant, from eggs received at random from the surrounding territory. Each sample assayed 45% egg solids, and nothing was added to the yolks prior to freezing. The samples were received in the Quartermaster Laboratory in excellent condition and kept stored at −20° F. during the course of the investigation. Samples were taken with sterile technique, and the phospholipides were extracted from the samples of frozen yolk in accordance with the official procedure (*2*).

Table I lists the per cent lipoid phosphoric acid content as determined during the 36-month storage study of the two samples of frozen egg yolks. The data given under the column headed "Proposed Method" was obtained during the developmental stages of the wet-ash colorimetric method. Three phases were involved in the development of the final procedure—the wet-ash technique, the colorimetry, and the combination of the two. The results reported were performed at progressive stages of completion of the method as noted in the table. From a study of Table I, which shows no significant difference in the lipoid phosphoric acid content of the two samples, two conclusions can readily be drawn—phospholipides do not deteriorate in egg yolks kept in freezer storage for 3 years, and the results obtained by the proposed method show less deviation than those obtained by the official procedure. Consequently, if egg noodles are found to be below contract requirements, the blame for the shortage in lipoid phosphoric acid cannot be put on the deterioration of the frozen yolk product, if the product has been kept under sanitary conditions in freezer storage. The discrepancy, then, would be caused by a shortage of yolk in the formulation, or deterioration of the manufactured stock during prolonged storage or storage under adverse conditions.

Another series of comparative determinations was made. As shown in Table II, the range for 9 results for the same sample of frozen yolk, obtained by the official method, was 1.92 to 2.09% lipoid phosphoric acid, and for 11 results by the wet-ash volumetric method, the range was 1.96 to 2.03%.

The results of a study, shown in Table III, made during the development of the colorimetric phase of this project gives similar values for lipoid phosphoric acid in frozen egg yolk.

The results obtained in the laboratory for lipoid phosphoric acid in frozen egg yolk are in agreement with the values obtained by Mitchell (*10*), who studied eggs from different parts of the country, and found that yolks separated from commercial fresh eggs gave average values of lipoid phosphoric acid ranging from 1.89 to 2.00%. The average of all results from 21 different localities was 1.94 %.

A study of a number of samples of noodles manufactured from frozen egg yolk (Table IV) shows that reliable results are obtained when the lipoid phosphoric acid content is determined by the proposed method. Comparison with values obtained by the official procedure shows values of the same magnitude with

less deviation from the mean. The values listed under "wet-ash volumetric" were obtained during the developmental stage of the proposed method when the wet-ash phase had been completed. The values listed under "wet-ash colorimetric" were obtained by the final procedure given herein. As the latest government specifications list egg requirements in terms of lipoid phosphoric acid without applying the 1.1 recovery factor the data given here for noodles have been computed on the same basis.

**Microbiology** Attention was given to the microbiology of frozen egg yolks. During the same time intervals that the lipoid phosphoric acid determinations were made, microbiological analyses were performed and the effect of freezer storage time on the microorganisms present in the frozen yolk was noted. The microbiological determinations included the standard plate count, coliforms, *E. coli*, mesophilic and thermophilic anaerobes, and yeasts and molds. Samples were drawn as prescribed by the American Public Health Association (*1*), thawed at room temperature for 20 to 30 minutes, and thoroughly mixed, and dilutions were prepared at once. The standard plate count, coliform, *E. coli*, and yeast and mold counts were made in accordance with the directions specified by the National Egg Products Association (*14*). The counts for mesophilic and thermophilic anaerobes were made using the serial dilution method, in liver infusion broth sealed with beeswax and vaseline, and incubated at 37° and 55° C. for 48 hours. Results of these studies agree, in general, with observations made by Winter, Burkart, and Wrinkle (*20*). These, and other workers in the field, have shown that a marked decrease in the standard plate count and coliform count occurs during freezer storage, and that most of this change occurs within the first few months of storage. From the results obtained in this experiment (Table V), it is concluded that the conditions prevailing in freezer storage are not conducive to microbiological action that would affect the deterioration of phospholipides within 3 years. Stiles and Bates (*17*) stored various grades of eggs at −10° F. for 1 year. Flasks of each product were removed at various time intervals and microbiological examination was made. Little variation in strictly fresh and commercially fresh frozen eggs was revealed during this period. Schneiter, Bartram, and Lepper (*15*) found that frozen eggs of good quality are able to withstand at least two complete thawings and refreezings without significant changes in microbiological content, and without acquiring abnormal appearance or color. It must therefore be assumed that the rapid decomposition of egg batter results from insanitary plant practices and improper processing, particularly when the freezing operation is delayed or prolonged.

## Conclusions

As an index of the egg content of noodles, lipoid phosphoric acid is a reliable determinant, provided that meticulous care is exercised in following the official procedure and that the method is applied on currently manufactured stock.

Phospholipides do not deteriorate appreciably in frozen egg yolk over a 36-month period in freezer storage, nor in noodles stored at room temperature during the first 6 months after manufacture.

Microbiological counts during freezer storage of egg yolk diminish over a period of 3 years, and the phospholipides in eggs of good quality are not affected by microorganisms under these conditions.

The lipoid phosphoric acid method has been streamlined by the use of a wet-ash digestion and instrumental method of measurement. Results for lipoid phosphoric acid in eggs and noodles, comparable to the official method, are obtained with relative ease and less technical skill by the proposed method.

Although the proposed "wet-ash colorimetric method" for the determination of lipoid phosphoric acid in noodles shows promise, its precision and accuracy must be proved through collaborative study prior to recommendation as an official method. The Quartermaster Subsistence Testing Laboratory suggests its use for screening purposes until the method can achieve official status.


## Acknowledgment

Grateful appreciation is expressed to Lt. Col. Earl G. Kingdon, V. C., Quartermaster Subsistence Testing Laboratory, Chicago Quartermaster Depot, for his continued support and assistance in the storage and methods development investigations made. It is with gratitude that the authors acknowledge the aid of Joan F. Fried for her performance of the microbiological studies conducted. Thanks are expressed to Leo Kaufman, who, as representative of Swift & Co., Union Stock Yards, Chicago, supervised the preparation of two uniform 30-pound


**Table V. Microbiological Counts in Frozen Egg Yolk During 3-Year Storage Study**

| Freezer Storage Time, Months at −20°F. | Microorganisms per Gram of Frozen Egg Yolk | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Standard Plate Count | | Coliforms | | E. Coli | | Mesophilic Anaerobes | | Thermophilic Anaerobes | | Yeasts and Molds | |
| | Sample A | Sample B | Sample A | Sample B | Sample A | Sample B | Sample A | Sample B | Sample A | Sample B | Sample A | Sample B |
| 1 | 250,000<br>350,000 | ... | ... | ... | 3,500 | ... | ... | ... | ... | ... | 100[a] | ... |
| 2 | 70,000<br>65,000 | ... | 10,000[a]<br>10,000[a] | ... | 7,500<br>6,900 | ... | 10,000 | ... | 1000 | ... | 100[a] | ... |
| 3 | 63,000<br>70,000 | ... | ... | ... | 9,700<br>13,000 | ... | 10,000 | ... | 1000[a] | ... | 100[a] | ... |
| 4 | 291,000<br>286,000 | ... | ... | ... | 20,800<br>20,900 | ... | 1,000[a] | ... | 100[a] | ... | 100[a] | ... |
| 17 | ... | 350,000<br>320,000 | ... | ... | ... | 1500<br>3100 | ... | ... | ... | ... | ... | 100[a] |
| 18 | ... | 126,000<br>100,000 | ... | 10,000[a] | ... | 3500<br>3500 | ... | 10,000 | ... | 1000 | ... | 100[a] |
| 19 | ... | 116,000<br>128,000 | ... | ... | ... | 1200<br>2100 | ... | 1,000[a] | ... | 1000 | ... | 100[a] |
| 20 | 31,000<br>47,000 | 254,000<br>230,000 | 10,000[a] | ... | 2,200<br>2,400 | 2400<br>4000 | 10,000 | 100 | 100[a] | 100[a] | ... | 100[a] |
| 36 | ... | 56,000<br>53,000 | ... | 1,000[a] | ... | 1200<br>1300 | ... | 100 | ... | 100[a] | ... | ... |

[a] Less than.

cans of frozen egg yolk given to this laboratory for this study. The assistance in the analytical work performed by Sander M. Goodman of this laboratory is appreciated. The authors are also grateful for the critical reviews of the manuscript made by C. W. Schroeder and James J. Winston.

### Literature Cited

(1) Am. Pub. Health Assoc., "Standard Methods for the Examination of Dairy Products," 9th ed., 1948.
(2) Assoc. Offic. Agr. Chemists, "Official Methods of Analysis," 7th ed., 1950.
(3) Buchanan, Ruth, *J. Assoc. Offic. Agr. Chemists*, **7,** 407 (1924).
(4) Fiske, C. H., and Subbarow, Yellapragada, *J. Biol. Chem.*, **66,** 375 (1925).
(5) Haenni, E. O., *J. Assoc. Offic. Agr. Chemists*, **24,** 119 (1941).
(6) Hertwig, Raymond, *Ibid.*, **7,** 91 (1923); **9,** 40 (1926).
(7) *Ibid.*, **36,** 61–2 (1953).
(8) *Ibid.*, p. 62.
(9) Koehn, R. C., and Collatz, F. A., *Ibid.*, **27,** 451 (1944).
(10) Mitchell, L. C., *Ibid.*, **15,** 282 (1932).
(11) *Ibid.*, **16,** 298 (1933).
(12) Munsey, V. E., *Ibid.*, **35,** 693 (1952).
(13) Munsey, V. E., U. S. Food and Drug Administration, Washington, D. C., private communication to Lt. Col. Earl G. Kingdon, V.C., Quartermaster Subsistence Testing Laboratory, Chicago Quartermaster Depot, October 1952.
(14) National Egg Products Assoc. Research Laboratory, Chicago, "Chemical and Bacteriological Methods for the Examination of Eggs and Egg Products," Dec. 29, 1949.
(15) Schneiter, R., Bartram, M. T., and Lepper, H. A., *J. Assoc. Offic. Agr. Chemists*, **26,** 172 (1943).
(16) Snell, F. D., and Snell, C. T., "Colorimetric Methods of Analysis," Vol. 1, p. 505, New York, D. Van Nostrand Co., 1936.
(17) Stiles, G. W., and Bates, C., U. S. Dept. Agr., *Bull.* **158** (1912).
(18) Tillmans, J., Riffart, H., and Kühn, A., *Z. Untersuch. Lebensm.*, **60,** 361 (1930).
(19) Winston, James J., Jacobs-Winston Lab., Inc., New York, N. Y., private communication to Lt. Col. Earl G. Kingdon, V.C., Quartermaster Subsistence Testing Laboratory, November 1950.
(20) Winter, A. R., Burkart, Blanche, and Wrinkle, Carolyn, *Poultry Sci.*, **30,** 372 (1951).

*Received for review March 16, 1953. Accepted July 9, 1953. Presented before the Division of Agricultural and Food Chemistry at the 123rd Meeting of the* AMERICAN CHEMICAL SOCIETY, *Los Angeles, Calif.*

**Corn Proteins Improved with Amino Acids**

---

## PROTEIN EFFICIENCY

# Improvement in Whole Yellow Corn with Lysine, Tryptophan, and Threonine

**BARNETT SURE**

**with the technical assistance of LESLIE EASTERLING, JOY DOWELL, and MARY CRUDUP, Department of Agricultural Chemistry, University of Arkansas, Fayetteville, Ark., and**

**The cooperation of NEVIN S. SCRIMSHAW, Institute of Nutrition of Central America and Panama, Guatemala City, Guatemala**

THAT THE PROTEINS IN CORN are deficient in lysine and tryptophan and that the proteins in wheat are deficient in lysine was found in 1932 by Mitchell and Smuts (*2*). The recent finding of Pecora and Hundley (*3*) that the proteins in white polished rice can be improved by the addition of threonine, and of Sure (*4*) that the proteins in whole wheat and in patent wheat flour can be further improved by threonine in presence of lysine and tryptophan, stimulated the study of the influence of threonine supplementation to the proteins in whole yellow corn. As a typical United States variety, the Staley Manufacturing Co., Decatur, Ill., supplied representative samples from 10 different cars, 95% of which was hybrid whole yellow corn.

Two typical Guatemala varieties of whole yellow corn, marked TGY and 142–48, were supplied by the Institute of Nutrition of Central America and Panama. Because in Guatemala 70% of the total proteins are derived from whole yellow corn, which are deficient biologically, it was of interest to determine whether they could be improved by addition of threonine in the presence of lysine and tryptophan.

This study was carried out on the Wistar strain albino rat; in all groups, which contained 12 animals in each