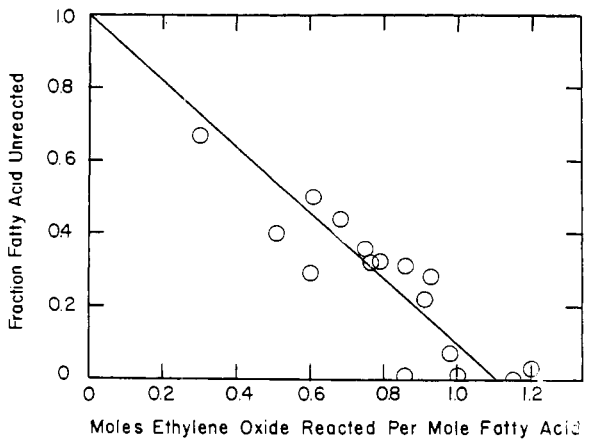

Figure 1. Mole fraction of free fatty acid in Myrj 45 reaction mixture

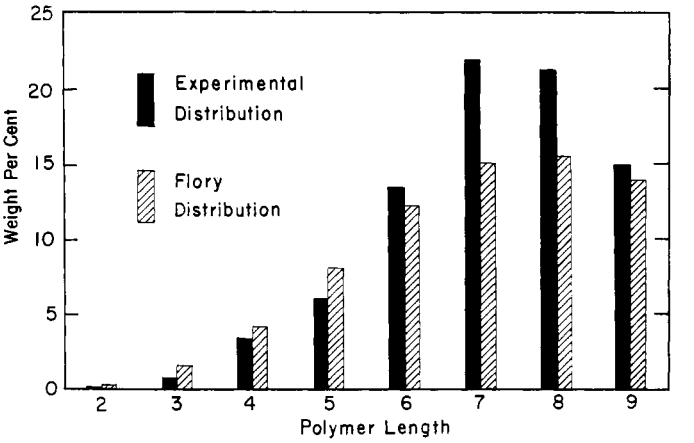

Figure 2. Experimental and theoretical polymer distributions of Myrj 45 polyglycols

cols; J. W. LeMaistre and L. A. Hartmann for devising the methylation procedure; and L. F. Gleysteen for helpful discussions of the data.

Literature Cited

(1) Corcoran, A. C., Page, I. H., *J. Biol. Chem.* **170,** 165 (1957).
(2) Flory, P. J., *J. Am. Chem. Soc.* **62,** 1561–5 (1940).
(3) Food Protection Committee, Food and Nutrition Board, National Academy of Science–National Research Council, Publ. **280,** 1953.
(4) Huntress, E. H., Mulliken, S. P., "Identification of Pure Organic Compounds," pp. 468–9, Wiley, New York, 1941.
(5) Karabinos, J. V., Quinn, E. J., *J. Am. Oil Chemists' Soc.* **33,** 223–5 (1956).
(6) Malkemus, J. D., Swan, J. D., *Ibid.*, **34,** 342–44 (1957).
(7) Mayhew, R. L., Hyatt, R. C., *Ibid.*, **29,** 357–62 (1952).
(8) Molina, E. C., "Poisson's Exponential Binomial Limit," p. 7, Van Nostrand, New York, 1942.
(9) Natta, G., Simonetta, M., *Rend. ist. lombardo sci.* **78,** No. 1, 336–46 (1945).
(10) Perry, S., Hibbert, H., *J. Am. Chem. Soc.* **62,** 2561–2 (1940).
(11) Shechter, L., Wynstra, J., *Ind. Eng. Chem.* **48,** 86–93 (1956).
(12) Weibull, B., Nycander, B., *Acta Chem. Scand.* **8,** 847–58 (1954).
(13) Wrigley, A. N., Smith, F. D., Stirton, A. J., *J. Am. Oil Chemists' Soc.* **34,** 39–43 (1957).

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CHOLINE MEASUREMENTS

Determination of Choline in Egg Products, Flour, and Noodles

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A colorimetric method is presented for determining choline in noodles as a measure of egg yolk content. The method measures the total choline of the phospholipides whether or not they have been altered by hydrolysis. Results are therefore independent of the manufacturing conditions and storage history of the samples. Typical analyses of dry whole eggs, commercial egg yolk solids, durum semolinas, and semolina-farina blends are reported.

THE EGG CONTENT of food products has been a subject of investigation in this country and in Europe for many years; yet there are no totally satisfactory methods of analysis.

The egg component of foods is most often estimated from an analysis of the lipide phosphorus content. However, manufacture and storage hydrolysis of phospholipides cause a transfer of phosphate from the fat-soluble phase to the fat-insoluble phase. The lipide phosphorus content is affected by processing and storage conditions of the sample.

Cholesterol provides a more reliable measure of egg content, but the complexity of some methods for determining cholesterol and the unreliability of others have prevented their broad application to food analysis problems.

This paper describes a colorimetric method for determining choline in noodles as an index of egg yolk content. Choline is combined with phosphate, or lipide phosphorus, in the lecithin molecule, but differs from it in that the solubility in the extraction solvent is not significantly affected by hydrolysis of the lecithin. The method therefore measures the total choline content of the phospholipides whether or not they have been altered by hydrolysis. This report also presents analyses of egg products and of flour and changes in the phospholipide fraction of noodles in storage under several conditions of temperature and relative humidity.

Methods

Special Apparatus and Reagents. Wiley mill, intermediate, equipped with 20-mesh screen (Fisher Scientific Co. No. 8-338A).

Soxhlet extraction apparatus, equipped with 250-ml. flasks, 40 × 205 mm. extractors, and 33 × 80 mm. extraction thimbles.

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Büchner-type glass funnels with fritted disks, fine porosity, 30-ml. capacity.

Reinecke salt (ammonium reineckate), 2% solution in absolute methanol, prepared fresh daily.

Procedure. Pass the sample of noodles through a Wiley mill equipped with 20-mesh screen. Weigh 10.00 grams of ground sample and 2 grams of filter-aid (Hyflo Super Cel, Johns-Manville diatomaceous earth) into an extraction thimble and mix thoroughly with a glass rod. For the analysis of dry whole egg and dry egg yolk use a 1.000-gram sample; for flour use 10.00 grams. Insert a cotton plug into the thimble, and extract continuously for 24 hours with approximately 100 ml. of absolute methanol, reagent grade.

Place the flask containing the methanol extract on a steam bath and evaporate it to a volume of approximately 5 ml. Add 30 ml. of saturated barium hydroxide solution (reagent grade), cover the flask with a small watch glass, and continue heating on the steam bath for an additional hour.

Rinse the cover glass with a small volume of distilled water, and collect the rinsings in the flask. Cool the flask to room temperature and acidify the contents with glacial acetic acid (reagent grade) to the phenolphthalein (1% alcoholic solution) end point. With the aid of a glass rod, filter through a folded 12.5-cm. Whatman No. 42 paper into a 50-ml. volumetric flask, and wash the extraction flask with three 5-ml. portions of distilled water. With the aid of the glass rod, add each of the washings to the funnel in such a manner as to wash down the sides of the filter. Dilute the combined filtrate and washings to 50 ml. with distilled water.

Pipet 10 ml. of the filtered solution into a 50-ml. Erlenmeyer flask. In the case of noodles containing 10% of whole egg solids instead of the customary 5.5% of egg yolk solids, it is preferable to dilute a 5-ml. aliquot with 5 ml. of distilled water. While swirling the flask, add with a pipet 5 ml. of ammonium reineckate solution. Stopper the flask with a rubber stopper and place it in a refrigerator at 4° C. for 18 hours. Also place a fritted-glass funnel of fine porosity and *n*-propyl alcohol (reagent grade) in the refrigerator.

Filter the precipitated choline reineckate onto the cold fritted glass funnel with the aid of a glass rod and suction. Allow the precipitate to drain dry and then wash the flask, funnel, and precipitate successively with three 2.5 ml. portions of cold *n*-propyl alcohol delivered from a pipet. With each addition of *n*-propyl alcohol to the funnel, mix the precipitate and alcohol with the glass rod. Leave the glass rod in the funnel for the rest of the procedure.

Suspend a 15-ml. graduated conical centrifuge tube in the suction flask with a piece of wire held by the rubber stopper in the neck of the flask. The stem of the funnel should extend a short distance into the tube. Add 2 to 3 ml. of acetone (reagent grade) to the Erlenmeyer flask to dissolve any remaining crystals of choline reineckate. Transfer to the funnel and mix the acetone and precipitate with the glass rod. Then apply very slight suction to drain the filter. Turn off the suction and repeat the solution with acetone 2 or 3 times more. There should then be no evidence of pink crystals in the flask or funnel, and the final volume of solution in the graduated tube should preferably be not more than 10 ml.

Dilute the solution in the graduated tube to 10 ml. with acetone, or if the volume is greater than 10 ml., record the volume. Stopper the tube with a rubber stopper and mix the contents by inverting the tube several times. Transfer the solution to a cuvette and measure the intensity of the color with reference to an acetone blank in a spectrophotometer or colorimeter at 530 mμ. In the case of a series of tests, complete each transfer and measurement before unstoppering the next tube.

Multiply the absorbance reading by the factor $\frac{\text{ml. of solution}}{10}$ and convert the corrected reading, A_c, to milligrams of choline by reference to a standard curve.

$$\text{Per cent choline} = \frac{\text{mg. choline} \times 5}{W \times V}$$

where W is weight of sample extracted in grams; and V is volume of aliquot taken for precipitation of choline reineckate in milliliters. In order to express results on a moisture-free basis, determine the solids content of separate portions of noodles, egg product, or flour by the vacuum oven method (*1*).

Preparation of Standard Curve. Weigh accurately approximately 2.3 grams of reagent grade choline chloride, dissolve in distilled water in a 100-ml. volumetric flask, and dilute to the mark. Determine the concentration of this solution by titrating 15 ml. with standard 0.1*N* silver nitrate solution. (One milliliter of 0.1*N* silver nitrate is equivalent to 12.12 mg. of choline.) Prepare a working solution by diluting exactly 5 ml. of the stock solution to 100 ml. with distilled water. Each milliliter of the working solution contains approximately 1 mg. of choline.

Pipet 0- to 9-ml. aliquots of the working solution, in 1-ml. increments, into 50-ml. Erlenmeyer flasks. Dilute each aliquot to 10 ml. with distilled water. Add 5 ml. of ammonium reineckate solution to each flask and continue with the procedure described previously. Prepare a standard curve relating absorbance to milligrams of choline.

Experimental

Extraction of Lipides. The extraction of lipides with methanol was tested for periods of 18, 24, and 36 hours. Recoveries from 5-gram samples of ground noodles reached a maximum within 18 hours, but 24 hours were required for 10-gram samples. The larger sample was specified in the procedure, however, because it provided optimum conditions of concentration and volume for the subsequent precipitation of choline reineckate. The completeness of the extraction of phospholipides from noodles and from dried egg yolk was further established by extracting them for 8 hours with petroleum ether after the customary 24-hour methanol extraction. There was no evidence of choline in the petroleum ether extracts when they were tested by the reineckate method.

Some investigators (*9*, *22*) have used alcohol-ether extraction for the determination of choline in animal tissue. Engel (*7*), in testing for choline in biological materials, and Rhian, Evans, and St. John (*28*), working with feeds, found methanol to be a more efficient solvent than alcohol-ether. They and others (*10*, *13–15*) used methanol in continuous extractors for the extraction of lipides from a variety of materials preparatory to the determination of choline. Successive extractions with alcohol and ether, according to the AOAC method (*3*) for lipides in noodles, were tried, but methanol extraction in a Soxhlet apparatus was adopted because it gave greater recovery of choline and with less manipulation.

Choline has been separated from animal tissue (*12*) and from salad cream and eggs (*5*) by hydrochloric acid hydrolysis rather than by solvent extraction of the lipides, but on noodles this produced a charred mass which was difficult to filter and results which were erratic.

Hydrolysis of Phospholipides. Phospholipides have been hydrolyzed with hydrochloric acid (*25*) and with potassium hydroxide (*15*) for the determination of choline. Barium hydroxide has been used more often for this purpose and was chosen here because of Glick's (*14*) statement that barium hydroxide removes betaine and other noncholine compounds, which would otherwise interfere with the ammonium reineckate reaction. One hour was adequate for complete hydrolysis and represented a savings of time over the periods commonly specified in other procedures.

Precipitation of Choline Reineckate. Published methods for choline cover a variety of materials and widely divergent findings have been reported regarding the optimum conditions of pH, temperature, and time for the precipitation of choline reineckate. The precipitation is accomplished in acid solution in

Table I. Recovery of Choline Added to Spaghetti and Macaroni

Choline Added to 10-G. Sample, Mg.	Choline Found, Mg.	Recovery of Choline, Mg.	Recovery of Choline, %
None	6.748	....	...
27.965	35.260	28.512	102.0
27.965	35.015	28.267	101.1
27.965	34.535	27.787	99.4
		Av.	100.8
None	6.605	....	...
28.085	35.015	28.410	101.2
28.085	35.500	28.895	102.9
28.085	34.535	27.930	99.4
		Av.	101.2

some methods (*9*, *15*) and in alkaline solution in others (*14*, *25*). Glick (*14*) claimed that precipitation in alkaline medium minimized interference by compounds other than choline. In the procedure described here, the hydrolyzed extracts of noodles are adjusted approximately to pH 8.3 before adding the Reinecke salt, whereas the choline chloride solutions used for preparing the standard curve have a pH of approximately 6.1. Variations in pH from 6 to 9 were found to have no effect upon the recovery of choline.

The time and temperature requirements for complete precipitation of choline reineckate have been reported to vary from a minimum condition of 10 minutes at room temperature (*29*) to overnight in the refrigerator (*5*, *7*, *10*, *22*). In this study, recoveries were always greater at 4° C. than at room temperature and at least 4 hours were required for maximum precipitation. As a matter of convenience, the precipitates were allowed to stand in the refrigerator overnight.

Colorimetric Estimation of Choline Reineckate. The intensity of the color of the acetone solution of choline reineckate did not change for periods up to 21 hours. In the calibration curve prepared with standard solutions of choline chloride, there was a linear relationship between color intensity and quantity of choline up to 9 mg. The regression line for the Coleman Universal spectrophotometer with 13-mm. square cuvettes, calculated by the method of least squares, was $9.632\ A_s - 0.028$ = mg. of choline in the final solution, in which A_s is the corrected absorbance of the solution at 530 mμ. Standard error of estimate was 0.050 mg.

Accuracy of Method. The efficiency of recovery of choline was tested by adding approximately 32 mg. of choline chloride, in water solution, to 10 grams of spaghetti or macaroni in a Soxhlet extraction thimble prior to extraction with methanol. The amount of choline added was determined by applying the reineckate method to other equal aliquots of the choline chloride solution. The recovery of added choline was approximately 101% (Table I).

The accuracy of the method was further tested by analyzing samples of known composition. Two samples of egg noodles were prepared on a laboratory scale and air dried at room temperature. Each consisted of approximately 350 grams; one contained 10.0% (moisture-free basis) of whole egg solids, the other, 5.5% (moisture-free basis) of commercial yolk solids. The ingredients (semolina-farina blends, dry whole egg, and dry egg yolk) and the finished noodles were analyzed for moisture and choline. The results of the choline analyses on the noodles were 100 and 104% of the expected values (Table II).

Results and Discussion

The foregoing results indicate that choline can be measured reliably by the reineckate method described.

Choline Content of Egg Products and of Flour. Table III shows the results of choline analyses of durum semolina, 50-50 blends of semolina and farina, dry whole egg, and dry commercial egg yolk. The averages of the results can be used as tentative values for estimating egg content, but a more extended survey, particularly of egg products, would be desirable. The uniformity of the flour samples suggests the applicability of the analytical results regardless of the proportions of durum semolina and farina in noodle products. Published values for egg products and flour are shown also for comparison.

Phospholipide Composition of Egg Products and of Flour. Inasmuch as choline is derived principally from lecithin, the choline–lipide phosphorus molecular ratio provides an approximation of the lecithin-cephalin distribution of the phospholipides of egg and flour. The figures for choline in Table III and values for lipide phosphorus derived from Hertwig (*20*) give a choline–lipide phosphorus mole ratio of approximately 0.75, for both products. This valve agrees with others reported for eggs (*23*) and for flour (*27*).

Egg Content of Noodles. The following expression relates the composition of egg noodles to choline content:

$$\text{Per cent egg component} = \frac{C - F}{E - F} \times 100$$

Table III. Choline Content of Flour and of Dried Egg Products

Product	Choline, Dry Basis, %		Av.
Current Results			
Durum Semolina			
Company A	0.069	0.069	0.069
Company B	0.071	0.074	0.073
Company C	0.073	0.072	0.073
		Av.	0.072
50-50 Semolina-Farina Blend			
Company A	0.073	0.069	0.071
Company B	0.071	0.071	0.071
Company C	0.076	0.072	0.074
Company D	0.073	0.072	0.073
		Av.	0.072
Dry whole egg			
Iowa	1.706	1.662	1.684
Missouri	1.704	1.709	1.707
Missouri	1.729	1.690	1.710
Missouri	1.712	1.737	1.725
Texas	1.645	1.599	1.622
		Av.	1.690
Dry commercial yolk			
Missouri	2.287	2.334	2.311
Missouri	2.369	2.419	2.394
Texas	2.278	2.278	2.278
Source unknown	2.305	2.259	2.282
		Av.	2.316
Published Values			
Flour	0.053 (*25*)		
	0.057 (*8*)		
	0.071 (*14*)		
	0.106–0.165 (*16*)		
Dry whole egg	1.54 (*25*)		
	1.6 (*5*)		
	1.80 (*17*)		
Dry commercial yolk	2.2 (*5*)		
	2.31 (*25*)		
	2.59[a] (*17*)		

[a] Not commercially prepared.

Table II. Choline Analyses on Samples of Known Composition

Composition: Component	Composition: Dry Basis, %	Choline Content, Dry Basis, %		Av.
Dry whole egg	10.0	1.712	1.737	1.725
Semolina-Farina blend	90.0	0.073	0.072	0.073
Finished noodles		0.237	0.239	
		0.237		0.238
		Calculated		0.238
Dry commercial yolk	5.5	2.369	2.419	2.394
Semolina-Farina blend	94.5	0.073	0.069	0.071
Finished noodles		0.205	0.205	
		0.207		0.206
		Calculated		0.199

where C is per cent choline in noodles; E is per cent choline in egg component; and F is per cent choline in flour component.

By substituting the average values from Table III for E and F the equation is resolved into the following:

$$\text{Per cent whole egg solids} = (C - 0.072)\ 61.80$$

$$\text{Per cent commercial egg yolk solid} = (C - 0.072)\ 44.56$$

Analysis of Noodles and Changes in Phospholipides during Storage. Hertwig (*19*), Buchanan (*4*) and others have reported on the incomplete recovery of lipide phosphorus from noodles. The high moisture content which exists during the manufacturing process promotes hydrolysis of the phospholipides and the consequent transfer of phosphate from the fat-soluble phase to the fat-insoluble phase. It is general practice to correct for this loss by adding 10% to the determined value for lipide phosphorus—an empirical factor first proposed by Hertwig (*20*).

There is a reduction of lipide phosphorus in noodles during storage also. Hertwig (*19*) reported a loss of 33% in only 6 days at room temperature under extreme conditions of humidity. Buchanan (*4*) reported losses of approximately 22% in 1 year under less severe conditions (approximately 9% moisture). Koehn and Collatz (*24*) showed losses of approximately 2% in 6 months but did not describe the storage conditions.

Because of the instability of the phospholipide fraction, interpretation of lipide phosphorus determinations for the purpose of estimating the composition of egg noodles is dependent upon the effects of the manufacturing and storage conditions of the samples. In addition, there are many operational difficulties in attempting accurate determinations of lipide phosphorus. These were reviewed comprehensively by Despaul, Weinstock, and Coleman (*6*).

The independence of the results from the storage history of the samples was established in a series of storage studies which were designed to gain information also on the effects of temperature and moisture on the stability of the phospholipides of noodles. In addition to choline and moisture determinations the following analyses were performed on the samples in these storage tests: lipide phosphorus, AOAC method for lipoid phosphoric acid (*2*); sterol, digitonin method (*26*).

Egg yolk noodles from a single lot were air packed and sealed in size 300 × 308 lacquered tin cans, approximately 4 ounces per can. They were stored at 40°, 70°, and 100° F. and one can at each temperature was withdrawn at each storage period (Figure 1). Because of the low moisture content—below 6%—the changes were insignificant at all temperatures.

A sample of whole egg noodles was ground to pass a 20-mesh screen in a Wiley intermediate mill. The ground material was transferred to glass jars which were capped and stored at 40°, 70°, and 100° F. (one jar at each temperature) (Figure 2). The moisture content varied little during 12 months of storage from the initial value of 8.5%. The only significant change was the decline in lipide phosphorus at 100° F. After 12 months, it was 83% of the initial value whereas the choline content after 12 months was 96% of the initial value.

Egg yolk noodles were manufactured and packaged in conformity with the requirements of the federal specification for noodles (*11*). The material was packed in waxed paper-lined fiberboard boxes—5 pounds to a box. The boxes were stored at 40°, 70°, and 100° F. and one box at each temperature was withdrawn at each storage period (Figure 3).

The initial moisture content of 11.4% was near the specification limit of 12.0%. It increased to 12.4% at 40° F., dropped slowly to 10.6% during 12 months at 70° F., and dropped rapidly to 6.5% during 12 months at 100° F. During the first 9 months, there were no significant trends in the data at any temperature except for the drop in lipide phosphorus at 100° F. to 77% of the initial value. The decrease in choline from the ninth to the 12th month at 70° and at 100° F. was disturbing to the supposition that the results would be independent of the age of the samples. However, the percentages of choline ranged more widely in this storage test than in the others—from 88 to 109% of the initial value.

There was no control over the selection of the samples which were part of a large procurement. A different box of noodles was analyzed each time and some of the variability in results may have been due to differences among samples. In order to check this point, unused portions of test samples which had been reserved in the refrigerator were analyzed for the percentage of sterols, which should remain unchanged (*18*, *24*). The sterol values also varied widely from 94 to 114% of the initial value and dropped in the period from

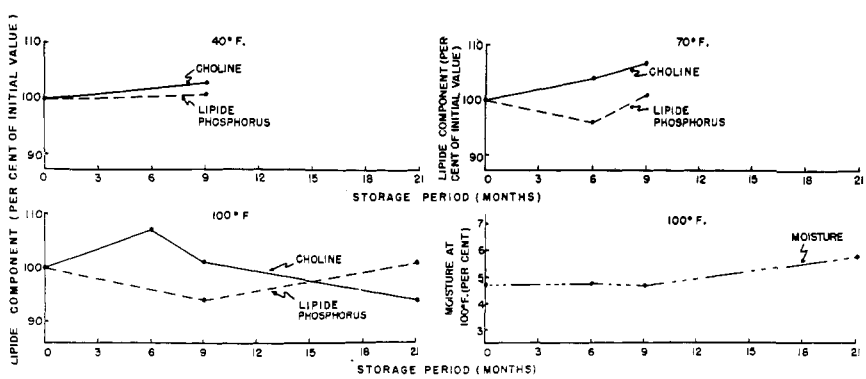

Figure 1. Analysis of egg noodles during storage; moisture content below 6.0%

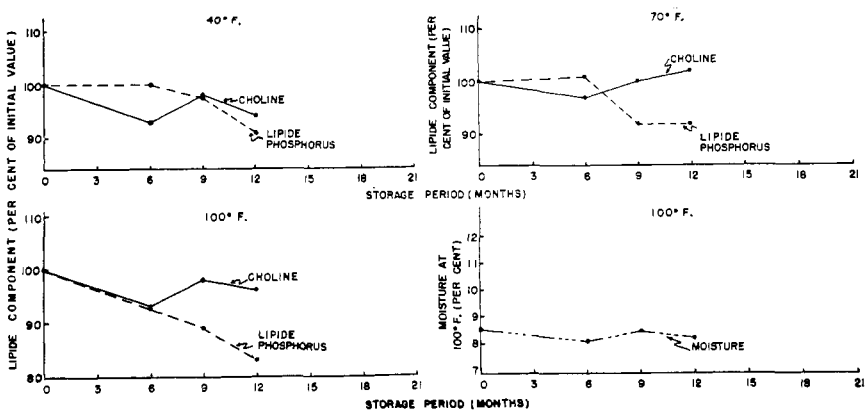

Figure 2. Analysis of egg noodles (20-mesh granulation) during storage; moisture content below 8.5%

9 to 12 months. These results also suggested that the variability was due, at least in part, to heterogeneous sampling. Furthermore, the choline and lipide phosphorus values after 18 months at 70° F. were higher than at 12 months.

In order to test the combined effect of high temperature and high humidity for an extended period of time, samples of noodles which contained 11.4% moisture were placed in open crystallizing dishes over saturated salt solutions in glass desiccators. Material held over saturated disodium hydrogen phosphate solution at room temperature (approximately 95% relative humidity) (*21*) became moldy within 3 months when the moisture content was 17%. Samples over saturated sodium nitrate at 95° F. (approximately 72% relative humidity) (*21*) reached a moisture content of 14% and became moldy in 2 months. Material stored over saturated cobaltous chloride solution at 95° F. (approximately 60% relative humidity) (*21*) maintained a uniform moisture content of approximately 11.5% and remained free of mold. Under these conditions, the lipide phosphorus content dropped to 68% of the initial amount in 6 months, but the choline content was 97% of the initial value after 13 months (Figure 4).

The storage tests demonstrated that the choline content of egg noodles was independent of the age of the samples. Even under conditions which promoted hydrolysis of the lecithin, as evidenced by the decrease in lipide phosphorus, the analyses for choline represented the entire choline content of the samples.

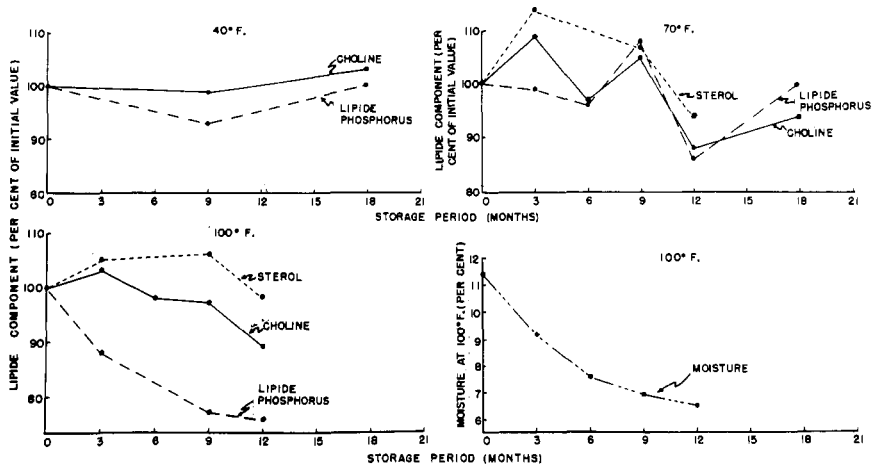

Figure 3. Analysis of egg noodles during storage; moisture content variable, starting at 11.4%

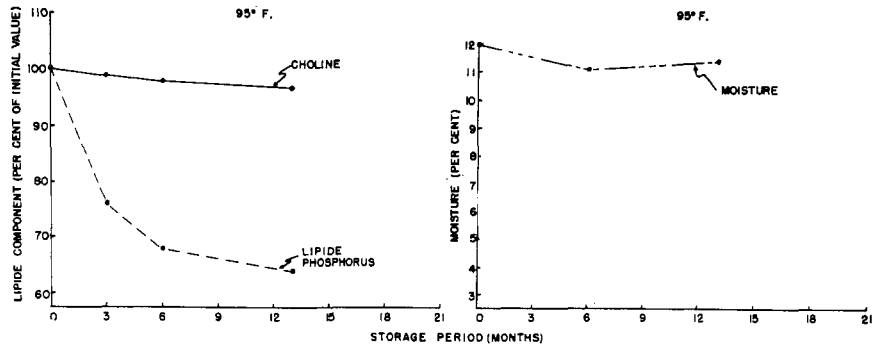

Figure 4. Analysis of egg noodles during storage; moisture content approximately 11.5%

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Literature Cited

(1) Assoc. Offic. Agr. Chemists, "Official Methods of Analysis," 7th ed., Sections 13.3, 13.98, and 16.3, pp. 192, 214, 275, 1950.
(2) *Ibid.*, Section 13.36, p. 202; *J. Assoc. Offic. Agr. Chemists* **36,** 76 (1953) (amended).
(3) Assoc. Offic. Agr. Chemists, "Official Methods of Analysis," 7th ed., Section 13.110, p. 215, 1950.
(4) Buchanan, Ruth, *J. Assoc. Offic. Agr. Chemists* **7,** 407–24 (1924).
(5) Daubney, C. G., Sexton, G. E. W., *Analyst* **75,** 305–9 (1950).
(6) Despaul, J. E., Weinstock, A., Coleman, C. H., J. AGR. FOOD CHEM. **1,** 621–6 (1953).
(7) Engel, R. W., *J. Biol. Chem.* **144,** 701–10 (1942).
(8) Engel, R. W., *J. Nutrition* **25,** 441–6 (1943).
(9) Entenman, C., Taurog, A., Chaikoff, I. L., *J. Biol. Chem.* **155,** 13–18 (1944).
(10) Evans, R. J., Davidson, J. A., *Poultry Sci.* **30,** 29–33 (1951).
(11) Federal Specification, Noodles, N-N-591a, 1951, and amendments.
(12) Fletcher, J. P., Best, C. H., Solandt, O. M., *Biochem. J.* **29,** 2278–84 (1935).
(13) Glick, D., *Cereal Chem.* **22,** 95–101 (1945).
(14) Glick, D., *J. Biol. Chem.* **156,** 643–51 (1944).
(15) Hack, M. H., *Ibid.*, **169,** 137–43 (1947).
(16) Hadorn, H., Jungkunz, R., *Mitt. Gebiete Lebensm. u Hyg.* **44,** 1–13 (1953).
(17) *Ibid.*, pp. 333–47.
(18) Haenni, E. O., *J. Assoc. Offic. Agr. Chemists* **24,** 119–47 (1941).
(19) Hertwig, R., *Ibid.*, **7,** 91–8 (1923).
(20) *Ibid.*, **8,** 107–19 (1924).
(21) International Critical Tables, Vol. 1, p. 67, McGraw-Hill, New York, 1926.
(22) Jacobi, H. P., Baumann, C. A., Meek, W. J., *J. Biol. Chem.* **138,** 571–81 (1941).
(23) Kaucher, M., Galbraith, H., Button, V., Williams, H. H., *Arch. Biochem.* **3,** 203–15 (1943).
(24) Koehn, R. C., Collatz, F. A., *J. Assoc. Offic. Agr. Chemists* **27,** 451–5 (1944).
(25) Munsey, V. E., *Ibid.*, **36,** 766–9 (1953).
(26) *Ibid.*, **37,** 92 (1954).
(27) Rewald, B., *Chem. & Ind. (London)* **1936,** pp. 1002–3.
(28) Rhian, M., Evans, R. J., St. John, J. L., *J. Nutrition* **25,** 1–5 (1943).
(29) Thornton, M. H., Broome, F. K., *Ind. Eng. Chem., Anal. Ed.* **14,** 39–41 (1942).

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