frozen samples. Nonfrozen samples in cans, irradiated at this level, were inferior in red color to the nonfrozen controls. Samples in cans, irradiated in the frozen state at this level, appeared to be slightly inferior in red color to the frozen controls.

At 100,000 rep., meat samples irradiated in the can appeared to have slightly better redness than nonirradiated controls for both frozen and nonfrozen samples. Irradiated nonfrozen samples in film packages appeared to have slightly less redness than controls during storage, but irradiated frozen samples had slightly more redness than controls. Red color is preserved better when irradiation is done in the frozen state.

Aureomycin. The data shown in Table III indicate that a dip into a water solution of aureomycin hydrochloride of either 8- or 15-p.p.m. concentration before packaging serves to decrease somewhat the redness of beef samples during storage. This may be due to the fact that certain genera of bacteria such as *Achromobacter* help maintain or return the red color of meat during storage (7). A wide-band antibiotic such as aureomycin inhibits bacteria.

Acknowledgment

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Literature Cited

(1) Ball, C. O., Clauss, W. E., Stier, E. F., *Food Technol.* **11,** 277 (1957).
(2) Bratzler, L. J., Proc. Seventh Research Conf., Council on Research, American Meat Institute, March 24–25, 1955.
(3) Brooks, J., *Biochem. J.* **23,** 1391 (1929).
(4) Clauss, W. E., Ball, C. O., Stier, E. F., *Food Technol.* **11,** 363 (1957).
(5) George, P., Stratmann, C. J., *Biochem. J.* **51,** 103 (1952).
(6) Grant, N. H., *Food Research* **20,** 250 (1955).
(7) Halleck, F. E., Ph.D. thesis, Rutgers University, New Brunswick, N. J., 1952.
(8) Landrock, A. H., Wallace, G. A., *Food Technol.* **9,** 194 (1955).
(9) Pirko, P. C., M.S. thesis, Iowa State College, Ames, Iowa, 1956.
(10) Rikert, John A., Ball, C. Olin, Stier, Elizabeth F., *Food Technol.* **11,** 520 (1957).
(11) Rikert, John A., Bressler, Lawrence, Ball, C. Olin, Stier, Elizabeth F., *Food Technol.* **11,** 567 (1957).
(12) *Ibid.,* p. 625.

FOOD ADDITIVE ANALYSIS

Composition of Polyoxyethylene (8) Stearate

**R. L. BIRKMEIER and J. D. BRANDNER**

Atlas Powder Co., Wilmington, Del.

The composition of polyoxyethylene (8) stearate was investigated to determine its safety as a food additive. This compound—made by reacting 7.4 moles of ethylene oxide per mole of commercial stearic acid—contains unesterified polyoxyethylene glycol and polyoxyethylene glycol monostearate, distearate in the molar proportions of 1.1 to 2.0 to 1.0, with free and esterified polyethylene glycols having equal polymer lengths. After allowance for small amounts of catalyst and water, the results are those expected if rapid ester interchange occurs. The polyglycols through the nonamer comprise 81% of the total polyglycols and the polymer distribution approximates a Poisson distribution.

POLYOXYETHYLENE (8) STEARATE (Myrj 45, Atlas Powder Co.) is made by reacting ethylene oxide with commercial stearic acid. The molar proportion of ethylene oxide to stearic acid is 8 to 1 if the stearic acid is assumed to be pure— i.e., to have a molecular weight of 284. As commercial stearic acid contains substantial proportions of fatty acids of lower molecular weight, such as palmitic and oleic, the acids used for the production of Myrj 45 have an average molecular weight of 270. In the manufacture of Myrj 45, 1.235 parts by weight of ethylene oxide react with one part of stearic acid, using 0.3% sodium methylate as catalyst. After reaction, the product is deodorized by heating under vacuum, bleached with hydrogen peroxide, filtered, and adjusted to 2.5 to 3.0% water.
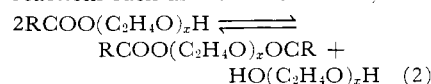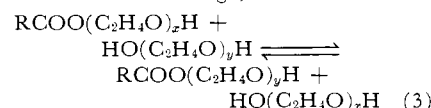
The general over-all reaction is:

$$RCOOH + xC_2H_4O \longrightarrow RCOO(C_2H_4O)_xH \quad (1)$$
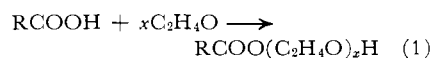
The final product contains polyoxyethylene chains of varying lengths with x averaging 7.4.

According to other investigators (11, 13), conditions are favorable to other reactions such as diester formation,

$$2RCOO(C_2H_4O)_xH \rightleftharpoons RCOO(C_2H_4O)_xOCR + HO(C_2H_4O)_xH \quad (2)$$

and ester interchange,

$$RCOO(C_2H_4O)_xH + HO(C_2H_4O)_yH \rightleftharpoons RCOO(C_2H_4O)_yH + HO(C_2H_4O)_xH \quad (3)$$

Myrj 45 is therefore expected to be a mixture of polyoxyethylene glycol monostearate, polyoxyethylene glycol distearate, and unesterified or "free" polyoxyethylene glycol. If Reactions 2 and 3

are rapid, the three species will have equal average polymer lengths and identical polymer distributions.

Separation of Myrj 45 into Unesterified Polyglycols and Esters

The unesterified, or free polyoxyethylene glycol in Myrj 45 is determined by a solvent extraction procedure. A sample of Myrj 45 is dissolved at room temperature in three times its weight of ethyl acetate. The solution is filtered to remove insoluble sodium stearate, present in Myrj 45 to the extent of 0.2 to 0.3%. The filtered solution is extracted four times with one fourth its volume of warm (70° C.) 10% aqueous sodium sulfate. The combined sodium sulfate extracts are washed twice with one fifth their volume of ethyl acetate. The sodium sulfate solution contains the free polyglycols; the combined ethyl acetate solutions contain the mixed monoesters and diesters.

The mixed esters are isolated by stripping off the bulk of the ethyl acetate under vacuum, and then removing the last traces of solvent and water by keeping at 45° C. and 0.5 mm. pressure for several hours. The sodium sulfate solution is evaporated to about 50% water, and the salts are precipitated with ethyl alcohol and filtered off. Free polyglycols are isolated by stripping off the ethyl alcohol and water and vacuum drying, as in the case of the esters.

The combined recoveries of polyglycols and mixed esters averaged 98.6% of theory.

The total polyglycols are obtained by saponification of Myrj 45. The esterified polyglycols are recovered by saponification of the mixed esters. A sample of the material to be saponified is added to one half its weight of water and enough 50% aqueous potassium hydroxide is added to provide a 50% excess over the amount required for complete saponification. The mixture is kept at 95° C., for 6 hours, cooled, acidified to pH 2 with 50% sulfuric acid, and kept at 75° C. for 1 hour. On standing, the mixture separates into an upper fatty acid layer and an aqueous layer containing salts and polyglycols. The fatty acid is extracted three times with one fourth its volume of hot water. The water washings are then combined with the aqueous layer and extracted three times with one fourth volume of low-boiling petroleum ether. The ether washings are discarded. The aqueous solution is then neutralized with aqueous potassium hydroxide. After evaporation to about 50% water, the polyglycols are separated from the water and salts, as described previously, for the unesterified polyglycols.

The yields of polyglycols average 98.4% of theoretical.

Determination of Free Polyglycol-Monoester-Diester Ratio

The weight fraction of free polyglycol in Myrj 45 is found by dividing the weight of recovered free polyglycol by the sum of the weights of recovered mixed esters and recovered free polyglycols. This method assumes any losses in the separation procedure to be proportionally divided between the polyglycols and esters. The average molecular weight of the free polyglycols used in converting weight fraction to mole fraction is calculated from their hydroxyl number. The relative amounts of monoester and diester are calculated from the saponification and hydroxyl numbers of the mixed ester portion after correcting for the small amounts of free fatty acid, ash, and water in the samples.

Two ways of calculating the relative amounts of monoester and diester in the mixed esters from their corrected ester and hydroxyl numbers were employed. In Method I, the weight fraction of monoester, m, is calculated from the ratio of observed hydroxyl number of the sample, OH, to the calculated hydroxyl number of monoester, OH_m, thus:

$$m = \frac{OH}{OH_m}$$

OH_m is calculated from the acid number of the fatty acid, and the hydroxyl number of the polyglycols obtained on saponification of the mixed esters.

In Method II, the mole ratio of monoester to diester, n, is obtained as follows:

$$n = \frac{2}{(E/OH - 1)}$$

E is the observed ester number of the mixed esters. The values reported for weight and mole fractions of monoester and diester were obtained by averaging results of both methods.

Determination of Polymer Distribution of Myrj 45 Polyglycols

The ethylene glycol content of Myrj 45 can be obtained by the chromotropic acid method of Corcoran and Page (1). The method is nonspecific and gives a positive test for any compound that can be oxidized to formaldehyde; however, no such compounds other than ethylene glycol are likely to be present in Myrj 45. Analysis of 13 lots gave total ethylene glycol contents of 0.04 to 0.08% based on Myrj 45.

Mono-, di-, and triethylene glycols may be estimated by the following procedure: Total polyglycols of Myrj 45, 2.5 liters, are mixed with 1/4 their weight of an immiscible, high-boiling mineral oil—i.e., Marcol GX or eicosane, and distilled through a 3-foot packed column. Distillation is continued at about 1-mm. pressure, until droplets of glycol no longer separate from the oil phase. (At this point the pot temperature is about 200° C.) The oil and polyglycol layers

of the distillate are separated and the polyglycol layer is washed twice with petroleum ether. The petroleum ether washings and the oil layer are extracted twice with water. Ethylene glycol is determined by chromotropic acid analysis of the polyglycol distillate, the water washings, and the material which has collected in the solid carbon dioxide trap during the distillation. The volatile glycols are then fractionated through a 3-foot Todd column at 1 mm. and the refractive index and hydroxyl numbers are determined.

Although the fractions were substantially constant-boiling, their refractive indices as compared with the data of Perry and Hibbert (10) and their hydroxyl numbers as compared with theoretical, indicated that most of the fractions were mixtures. On the assumption that each fraction consisted of only two adjacent homologs, the quantities of ethylene, diethylene, and triethylene glycol were calculated. The ethylene glycol content of the distillate fractions by chromotropic analysis agree reasonably well with the amount calculated from refractive index and hydroxyl value. Ethylene glycol was positively identified on a fraction estimated to contain 86% ethylene glycol by preparing the dibenzoate (melting point 73° C.) and comparing it with an authentic sample (4).

The trimer through nonamer distribution of Myrj 45 total polyglycols may be determined by fractional distillation of their dimethyl ethers. The total polyglycols, which are obtained by saponification of Myrj 45 as previously described, cannot be distilled directly as the temperatures required for distillation of the hexamer and higher polymers cause considerable thermal decomposition to take place even at pressures of 1 mm. or less. Methylation lowers the boiling points of the polyglycols by about 40° C. and improves their resistance to pyrolysis. Polymers higher than the nonamer could not be distilled because of thermal decomposition.

The dimethyl ethers of the total polyglycols from two batches of Myrj 45 were fractionated in a 3-foot spinning band column at a pressure of 2 mm.—nine fractionations are carried out. Distillate fractions were analyzed by means of refractive index using a Bausch & Lomb precision refractometer, which gives refractive index reliable to three units in the fifth decimal place. Each distillate fraction was assumed to contain two components, the relative amounts of which are found by linear interpolation of refractive index. A calibration curve of refractive index vs. polymer length of the polyglycol dimethyl ethers was obtained.

Methylation of Polyglycols

The following procedure converts

polyglycols to their corresponding dimethyl ethers: For each mole of polyglycol, 8 moles of sodium hydroxide are added as a 30% aqueous solution and 4 moles of dimethyl sulfate are added slowly. The reaction temperature is maintained at 32° to 35° C. The reaction mixture is then kept at 75° C. for 2 hours, and 3 moles of ammonium hydroxide are added as a 30% aqueous solution to destroy excess dimethyl sulfate. The mixture is stirred at 35° C. for several hours to drive off amines and excess ammonia. On cooling, the mixture separates into an upper layer, containing most of the product, and a lower aqueous layer, containing salts and a small amount of product. The two layers are neutralized separately with 10% sulfuric acid. The upper layer is extracted four times with benzene. The raffinate is concentrated to $1/3$ of its volume, filtered, and extracted several more times with benzene. The lower layer of the reaction mixture, after neutralization, is concentrated to $1/4$ its volume and extracted several times with benzene. All the extracts are combined and stripped of benzene.

To determine whether the methylation procedure is quantitative, crude tetraethylene glycol was isolated from Carbowax 200 (Union Carbide Corp.), a mixture of polyoxyethylene glycols of average molecular weight 200, by distillation through a 6-inch Vigreux column. The crude material was then fractionated twice in a 4-foot column containing Cannon packing. The fraction which was methylated had a boiling range of less than 1° C. at 2 mm. and a hydroxyl number of 573 (theoretical = 578). The product, tetraethylene glycol dimethyl ether, was isolated in 97.5% yield. On fractionation in the Cannon-packed column, 98.8% distilled over in a range of 1° C. and at a constant refractive index (n_D^{20}) of 1.4324. The product had the following analysis before distillation: ash = 0.05%; hydroxyl number = 0.0; acid number = 0.2.

Refractive Index Standards

Refractive index was used to analyze the distillate fractions obtained on fractionating the dimethyl ethers of Myrj 45 total polyglycols to determine their polymer distribution. The literature does not give data on the refractive indices of the dimethyl ethers of polyglycols beyond the tetramer. Refractive index standards were obtained in three ways.

Samples of trimer and tetramer, available commercially from the Ansul Chemical Co., were fractionated in a 3-foot spinning band column, and the middle cuts were taken as refractive index standards.

After the fractionations of the dimethyl ethers of Myrj 45 polyglycols had been completed, substantially constant-boiling fractions of constant refractive index, believed to be the hexamer, heptamer, octamer, and nonamer, respectively, were distilled in the 3-foot spinning band column, and the middle cuts of each were redistilled through the same column. The twice redistilled samples were assumed to be pure dimethyl ethers of hexa-, hepta-, octa-, and nonaethylene glycol. Not enough pentamer was available for purification in this manner.

Samples of hexamer and octamer were synthesized and their refractive indices were in good agreement with the values in the second method. The octamer, $CH_3O(CH_2CH_2O)_8CH_3$, was prepared by reaction of dichlorodiethyl ether with the sodium salt of triethylene glycol monomethyl ether. The latter was prepared from triethylene glycol by reaction with an equimolar amount of dimethyl sulfate. The glycol monomethyl ether was separated from the dimethyl ether and unreacted glycol by fractional distillation and analyzed as: $n_D^{20} = 1.4382$; hydroxyl number = 342 (theoretical = 341); and boiling point = 88–89° C. at 2.0 mm. Sodium dispersion in toluene was added to the monomethyl ether to prepare the sodium salt. Freshly distilled dichlorodiethyl ether was then added dropwise. The toluene was stripped, under vacuum, and the precipitated sodium chloride was filtered off. Octaethylene glycol dimethyl ether was isolated in 15% yield, based on starting glycol, by fractional distillation. The middle cut was redistilled and the following analysis was obtained: hydroxyl number = 0.0; iodine number = 0.0; chlorine by sodium fusion, negative; and water = 0.02%, $n_D^{20} = 1.4499$. Hexaethylene glycol dimethyl ether was prepared

in an analogous manner using diethylene glycol as starting material.

When the refractive indices of the polyglycol dimethyl ethers, obtained as above, are plotted against the reciprocal of polymer length, the points fall on a smooth curve. The indices for the pentamer and decamer were obtained from this curve by interpolation and extrapolation (Table I).

Discussion

The hydroxyl numbers of the free, esterified, and total polyglycols of several batches of Myrj 45 (Table II), indicate that average molecular weights of polyglycols are identical within the limits of experimental error ($\pm 2\%$). These numbers correspond to average molecular weights of 337 to 349 and average polymer lengths of 7.25 to 7.50.

As the free and esterified polyglycols have the same average polymer length, rapid ester interchange must be occurring. Table III gives the experimental mole ratios and weight percentages of free polyglycol, monoester, and diester as determined on four plant batches.

The report (3) of the Food Protection Committee, Food and Nutrition Board on the safety of polyoxyethylene stearate in Myrj 45 indicates the presence of approximately equimolar quantities of the three types of constituents. A review of information submitted for inclusion indicates an error in calculation of the proportions of the three constituents. Previously determined ester and hydroxyl numbers, utilized to recalculate the proportions of free polyol, monoester, and diester by Method II, yielded the follow-

Table I. Refractive Indices of Polyoxyethylene Glycol Dimethyl Ethers

Polymer	n_D^{20}
3	1.42333
4	1.43245
5	1.43892
6	1.44373
7	1.44724
8	1.44991
9	1.45215
10	1.4540

Table II. Hydroxyl Numbers of Myrj Polyglycols

Batch of Myrj	Free Polyols	Esterified Polyols	Total Polyols
A	a	a	322
B	330	327	328
C	322	a	a
D	330	a	a
E	333	a	a

a Not recovered.

Table III. Composition of Myrj 45[a]

Batch of Myrj 45	Weight Per Cent			Mole Ratio		
	Free polyglycol	Mono-ester	Di-ester	Free polyglycol	Mono-ester	Di-ester
A	16.3	48.4	35.3	1.15	1.96	1.00
B	15.7	49.0	35.3	1.11	1.98	1.00
C	15.4	48.8	35.8	1.07	1.95	1.00
D	15.9	49.6	34.5	1.15	2.05	1.00
Av.	15.8	49.0	35.2	1.12	1.99	1.00

a On dry, ash-free, free fatty acid-free basis.
Myrj 45, as marketed, contains 2.5–3.0% water, 0.2% ash, and 0.16% free fatty acid.

ing range of values: free polyol = 15.7 to 18.4%; monoester = 37.8 to 47.2%; and diester = 36.8 to 43.8%.

Although the earlier measurements were not as precise, a comparison of the above percentages with the more accurate data in Table III shows good general agreement.

As rapid ester interchange is occurring, it can be shown statistically that, if the only reactants were ethylene oxide and fatty acid, the product should contain free polyglycol, monoester, and diester in 1 to 2 to 1 molar proportions. The experimental ratio of monoester to diester given in Table III agrees with theory, while the amount of free polyglycol is significantly greater than theoretical. The determined average mole ratio of 1.12 to 1.99 to 1.00 corresponds with 0.030 mole of excess polyglycol per mole of fatty acid. The presence of an excess of polyol is confirmed by the fact that the hydroxyl number of Myrj 45 is greater than the ester number. For the batches analyzed, the average hydroxyl number is 98.6 and the ester number, 92.5. If the mole ratio were exactly 1 to 2 to 1, the hydroxyl number would equal the ester number.

From the weight proportions of ethylene oxide, fatty acid, and catalyst, which are combined, and from the saponification number of the fatty acid, the saponification number of the product can be calculated merely by dilution. The calculated saponification number is 92.6—in excellent agreement with the average observed ester number of 92.5. If there were no other reactants, the hydroxyl number would also be 92.6. The sodium methylate catalyst is used at a level of 0.032 mole per mole of fatty acid, and thus may contribute a maximum of three points to the hydroxyl number of the product. This leaves, unaccounted for, three points of the observed hydroxyl number. Water, 0.05%, in the fatty acid and ethylene oxide, if converted to polyol, accounts for this discrepancy.

During the preparation of Myrj 45, a minor proportion of the ethylene oxide is reacting with materials other than fatty acid. When allowance is made for the reaction of ethylene oxide with water and catalyst, the average chain length of the polyoxyethylene glycol turns out to be about 7.4.

Malkemus and Swan (6) have recently published a method of analysis of polyethylene glycol esters, which they have applied to products made by esterifying polyethylene glycol with fatty acid and to adducts of ethylene oxide and fatty acid. Free polyglycol is extracted from the product by saturated salt solution. The polyglycol is not recovered, but the ester portion is analyzed for saponification number and hydroxyl number. From these analytical constants and those of the original material, the quan-

tities of the three constituents are calculated. This procedure is simple, but requires very high precision in the determination of the analytical constants. The procedure reported here, involving the recovery and analysis both of the polyols and of the esters, allows determination of the hydroxyl value and average molecular weight of the free polyols, and analysis of the separated polyol for any ester carried over into the aqueous phase, thus enabling a correction to be made for the weight of free polyol.

Flory (2) has deduced a relationship by means of which the mole fraction of each polymer can be calculated if two assumptions are made: that the number of propagating molecules, N° remains constant throughout the polymerization and that the rate constant for addition of ethylene oxide to all polymers is equal regardless of polymer length. This leads to the expression $N_x/N^\circ = e^{-v} v^{x-1}/(x - 1)!$, where N_x is the number of molecules containing $x - 1$ ethylene oxide units and v is the ratio of the number of ethylene oxide molecules consumed to the total number of propagating molecules. This is a Poisson distribution (8) and several investigators (5, 7) have reported it to apply to ethylene oxide additions within the accuracy of their fractionations.

The mole ratio of ethylene oxide to stearic acid in Myrj 45 is 7.4. This value is not satisfactory for v in the Flory formula, as the addition of ethylene oxide to stearic acid takes place before any significant addition to ethylene glycol monostearate. Figure 1 gives the mole fraction of free fatty acid in the Myrj 45 reaction mixture as a function of the moles of ethylene oxide reacted per mole of original fatty acid. The data for this graph were calculated from the acid numbers of samples withdrawn from the autoclave during the manufacture of Myrj 45 after various known amounts of ethylene oxide had reacted. The mole fraction of free fatty acid falls practically linearly from one to zero, during the reaction of the first mole of ethylene oxide per mole of fatty acid, indicating, as has been demonstrated previously (11, 13), that the preferred reaction is between fatty acid and ethylene oxide. If stearic acid and ethylene glycol monostearate had identical reactivities toward ethylene oxide, a calculation of the Poisson distribution shows that 32% of the fatty acid would remain after the reaction of one mole of ethylene oxide. The Flory distribution for Myrj 45 is therefore calculated on the basis that 6.4 moles of ethylene oxide are added to one mole of ethylene glycol monostearate.

The experimental polymer distributions obtained by averaging the data for nine fractional distillations of two batches of Myrj 45 polyglycols are given in

Table IV. Polymer Distribution of Myrj 45 Total Polyols

Polymer	Weight %	Average Deviation
1	0.05	±0.015
2	0.10	. . .
3	0.38	±0.15
4	1.9	±0.25
5	3.4	±0.35
6	7.8	±0.6
7	12.7	±1.2
8	12.4	±0.9
9	8.6	±0.8
10 and above[a]	10.6	

[a] By difference.

Table IV. Values are reported as weight per cent of Myrj 45. Values in the average deviation column give an indication of the reliability of the data. The quantities of ethylene through hexaethylene glycol given in Table IV are in substantial agreement with the data of the Food Protection Committee report (3). The procedure of separating the polyols as their dimethyl ethers is superior to the molecular distillation of the polyols employed previously. Reliable data on heptaethylene glycol and higher polyethylene glycols are not obtainable by molecular distillation.

In Figure 2 are plotted the experimental distribution of total polyglycols and the distribution calculated by means of the Flory equation. The values in the figure are as weight per cent of polyglycols and may be converted to weight per cent of Myrj 45 by multiplying by 0.58. The similarity of the experimental and theoretical polymer distributions is apparent. However, the differences in the individual values are greater than can be accounted for by errors in the fractional distillations—being of the order of several standard deviations. The discrepancies are probably due to the fact that not all polymers are equally reactive toward ethylene oxide. If the rate constants for the addition of ethylene oxide to polyglycol stearates decrease slightly with increasing polymer length, a distribution similar to the experimental distribution and having a sharper peak than the Flory distribution would be obtained. An unsuccessful attempt was made to apply the generalized equation of Natta and Simonetta (9) for the case where each polymer reacts at a different rate. The simplifying assumption of Weibull and Nycander (12), that the rates of all additions after the first are equal, was insufficient to explain the results presented here.
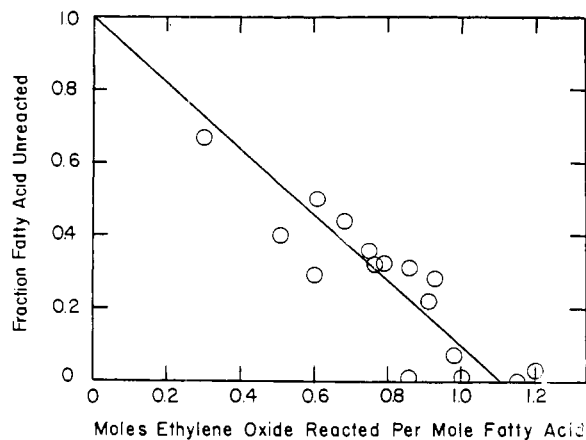
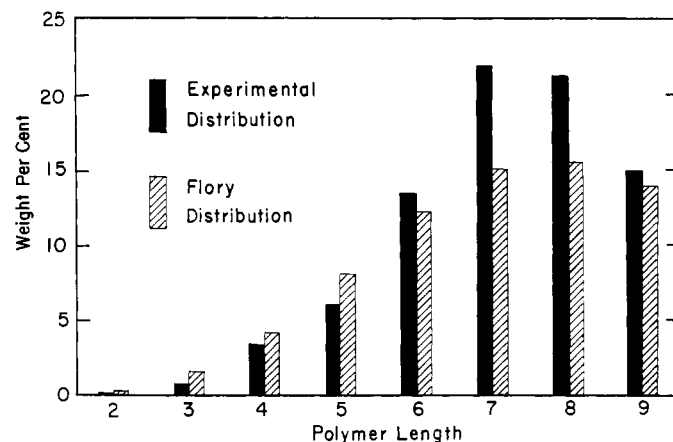Figure 1. Mole fraction of free fatty acid in Myrj 45 reaction mixture

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).

CHOLINE MEASUREMENTS

Determination of Choline in Egg Products, Flour, and Noodles

HAROLD SALWIN, MARY D. DEVINE[1], and J. H. MITCHELL, Jr.[2]

Quartermaster Food and Container Institute for the Armed Forces, 1819 West Pershing Rd., Chicago, Ill.

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.

[1] Present address, 8615 South Kenton Ave., Chicago, Ill.
[2] Present address, Clemson Agricultural College, Clemson, S. C.