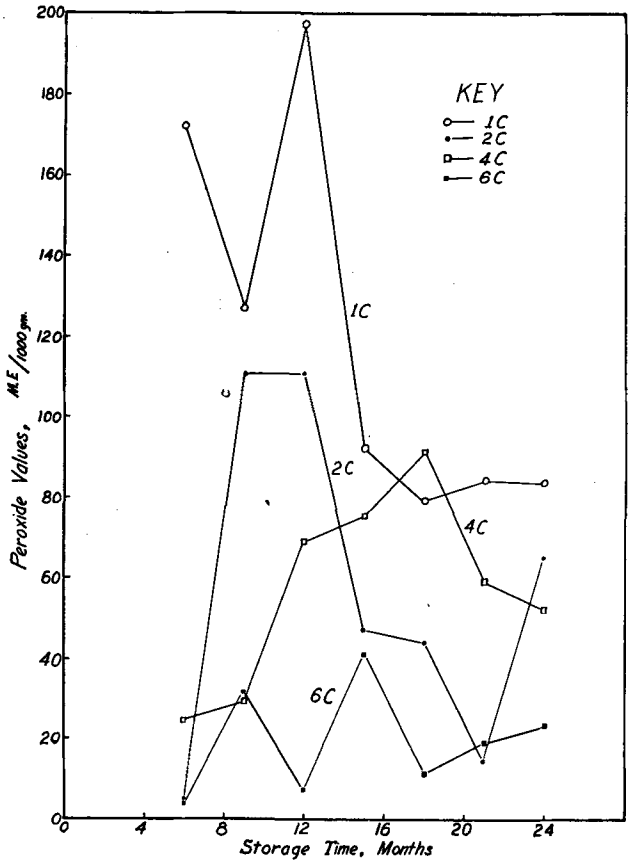

FIG. 10. Rate of peroxide formation in Type I biscuits stored in sealed cans at 100°F.

and 6). Where storage conditions require a hermetically sealed container, the use of the Rancimeter method for ration biscuits would appear to be of questionable value in estimating relative shelf life.

Summary

The comparative rates of oxidation of the shortening in 22 lots of biscuits held under three different conditions of packaging at two different storage temperatures have been discussed. Relating stability tests to storage tests is practically impossible considering the numerous conditions of storage which might be encountered. Accelerated tests, however, are valuable for comparative purposes.

Considering only the development of "rancidity," a 100-hour shortening appears to be adequate protection up to 12 months of storage at a maximum temperature of 100°F. for either Type I or Type IV Army ration biscuits when stored in either sealed cans or adequate breather-type containers.

The poor storage life of Army ration biscuits, packaged in fiberboard containers, was shown to be due primarily to the nature of the packaging material.

Insofar as Army ration biscuits are concerned, the addition of N.D.G.A. to lard does not result in a significant increase in biscuit stability.

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The Estimation of Glycerol, Diglycerol, and Polyglycerol in Commercial Diglycerol*

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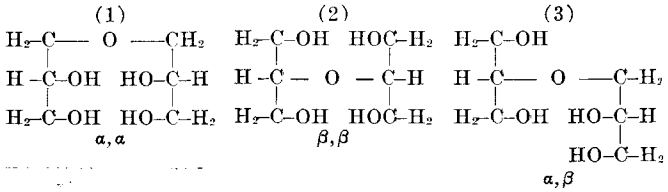
WHEN glycerol is polymerized by any of the methods given in the literature, the polymerization results in a series of compounds containing two or more glyceryl residues. The commercial "Diglycerol" that we are interested in is a polymer containing small amounts of glycerol, large amounts of dimeric polymer, and varying amounts of material containing three or more glyceryl residues. As the material is hygroscopic, varying amounts of water are also present.

Although chemical and physical methods of analysis are available for the binary mixture glycerol-diglycerol (1), no chemical method for a mixture containing higher polymers is available.

The Problem

Since glycerol has three hydroxyl groups, any of which takes part in the etherification reaction,



it is obvious that the three di-isomers as schematically outlined above may theoretically be formed. The presence of β,β diglycerol is improbable since activation of the β-hydroxyl group is required. The addition of another glyceryl residue to form triglycerol results in a large increase in the number of isomers, while the addition of two glyceryl residues to form tetra-glycerol results in an even larger number of isomers. Various cyclic polymers may also be formed to increase the number of individuals, but no evidence of low molecular weight compounds of this type has been found.

All these compounds have the same functional

* Presented at 39th Annual Meeting, American Oil Chemists' Society, May 4-6, 1948, New Orleans, La.

groups useful for analytical procedures making a normal analysis for each ingredient impractical. Acetylation and dichromate oxidation reactions, although quantitatively used for glycerol, are inadequate when applied to the polymers due to steric hindrance factors. If, however, the mixture is considered to contain three individuals, such as glycerol, diglycerol (all possible isomers), and polyglycerol (all remaining polymers), a method for estimating each by the use of periodic acid can be realized.

Principle of the Method

The water normally present in technical diglycerol does not influence the periodic acid oxidation reaction. It can be separately determined and a correction factor applied to determinations made on the material as received, saving the work usually necessary with hygroscopic materials.

The action of periodic acid upon compounds containing adjacent hydroxyl groups has in recent years been extensively studied and employed in various analyses and syntheses (2, 3, 4, 5, 6). The reaction between adjacent dihydroxy compounds of the type here involved follows the general equation:

$$\begin{array}{l} CH_2{-}OH \\ | \\ (CHOH)_n + (n+1)HIO_4 \rightarrow nHCOOH + 2HCHO + (n+1)HIO_3 \\ | \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad + H_2O \\ CH_2OH \end{array}$$

Thus glycerol with periodic acid is oxidized to formaldehyde and formic acid. The formic acid can be determined by acidimetry and so long as no other compound containing three adjacent hydroxyl groups has been simultaneously oxidized, the value obtained will be a quantitative measure of the glycerol present.

The α,α-diglycerol reacts with two moles of periodic acid to give a di-aldehyde and formaldehyde

$$CH_2OH{-}CHOH{-}CH_2{-}O{-}CH_2{-}CHOH{-}CH_2OH \longrightarrow 2HCHO + CHO{-}CH_2{-}O{-}CH_2{-}CHO$$

while the α,β form reacts with one mole to give an hydroxyaldehyde and formaldehyde

$$CH_2OH{-}CHOH{-}CH_2{-}O{-}CH(CH_2OH)_2 \longrightarrow HCHO + CHO{-}CH_2{-}O{-}CH(CH_2OH)_2$$

These are typical of all polymerized glycerol compounds which oxidize to give formaldehyde plus complex aldehydes. In no case is there any possibility of formic acid production as the third hydroxyl group has been consumed in the etherification reaction. Thus each polymer will react with a definite number of equivalents of oxidant and so if consistent mixtures of isomers are found in the diglycerol and polyglycerol fractions, definite oxidant equivalents can be determined for each of them.

When such equivalents are known and are plotted on triangular co-ordinates so that the sum of the three components is 100 per cent, a determination of one component, glycerol, and an oxidation value for the total are all that is necessary to locate the mixture on the plot.

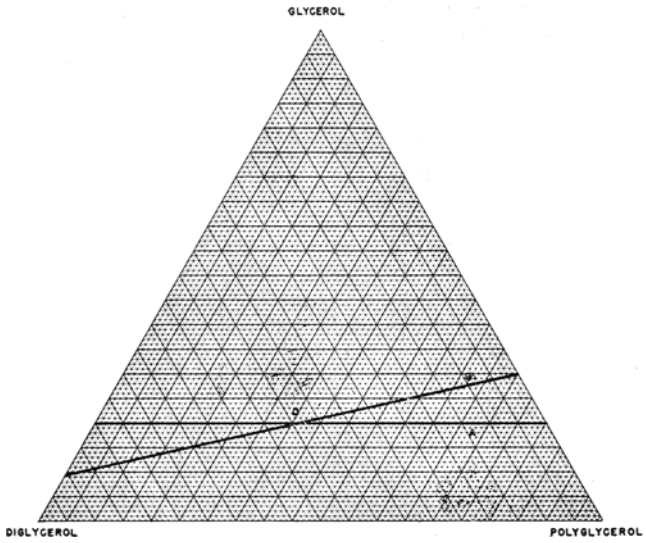


FIG. 1.

In Fig. 1 these eequivalents are calculated as "total oxidizable as % glycerol" for each of our three components—glycerol, diglycerol, and polyglycerol. The apexes of the graph represent the various components. If line (a) represents the per cent glycerol in the sample and line (b) is a tie line representing the "total oxidation value calculated as % glycerol" then the intersection of the lines gives the relative composition of the mixture.

Experimental

The time necessary for complete oxidation of a polymer mixture is shown in Fig. 2. Equal aliquots were oxidized with periodic acid and the resulting mixture analyzed at the times indicated, showing that one hour is sufficient for complete reaction.

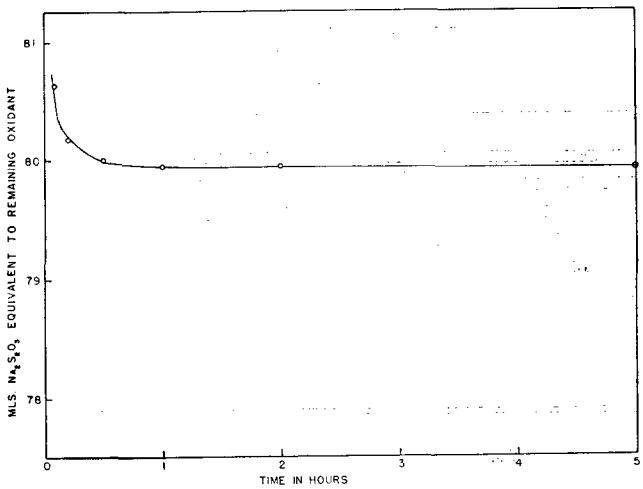


FIG. 2.

Previous work with the periodate oxidation (6) had shown that approximately a 5:1 molar ratio of oxidant to polyalcohol was necessary for complete oxidation and this is true in the present case.

Oxidation equivalents were determined on a series of diglycerols and polyglycerols prepared by careful fractional distillation. The polyglycerol fraction is the distillation residue from the diglycerol distilla-

tion. Attempts to further purify this material results in decomposition so the oxidation values were determined directly. Table I shows the results obtained.

TABLE I

Oxidation Equivalents (Glycerol = 100)

Sample No.	Diglycerol as "Oxidizable as % Glycerol"	Deviation from the Mean	Polyglycerol as "Oxidizable as % Glycerol"	Deviation from the Mean
1	45.28	+0.05	28.88	—0.12
2	45.67	+0.44	28.51	—0.49
3	45.51	+0.28	28.49	—0.51
4	45.39	+0.16	29.09	+0.09
5	44.80	—0.43	29.09	+0.09
6	45.00	—0.23	29.82	+0.82
7	44.97	—0.26	29.11	+0.11
8	45.22	—0.01	28.95	—0.05
Average	45.23	±0.23	29.00	±0.29

These results are easily within the probable variation in polymerization to give molecules requiring different oxidation equivalents.

The determination of glycerol by oxidation with periodic acid and the titration of the resulting formic acid has been reported (3). Using an aqueous oxidizing solution so that only inorganic acids would be present with the formic acid, analyses were made on various mixtures of glycerol and diglycerol for glycerol content. The results are shown in Fig. 3. The effectiveness of the aqueous reagent in relation to the acetic acid oxidant was also tested and results showed they were equivalent in reaction. The various points in Fig. 3 were determined by graphing each determination separately and choosing the correct equivalence end-points in each case. This variance of pH at the end-point makes the method impractical for this analysis when the fraction of glycerol to polymer is changeable and small.

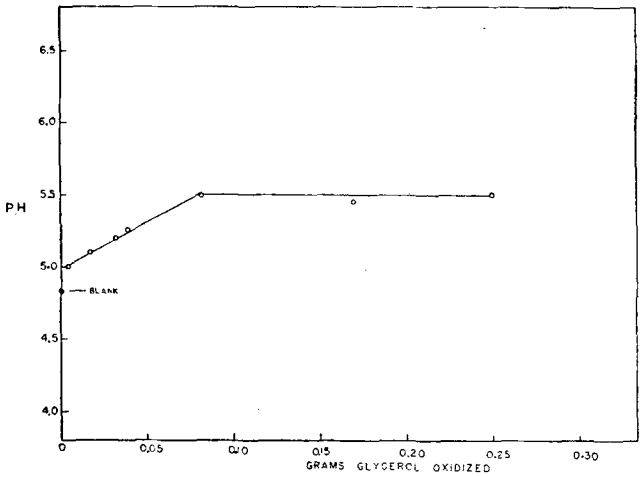


FIG. 3.

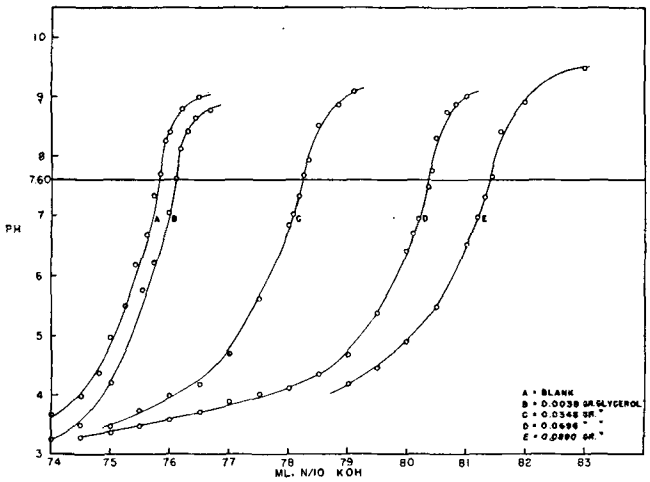


FIG. 4.

Consideration of these data led to the thought that the unused periodic acid may have some buffering effect in this pH range. The different results found may be a result of the different ratios of formic to periodic acid in the various samples. Thus a sample containing a small fraction of the total as glycerol is oxidized with nearly the same total of periodic acid as one containing only glycerol. In the first case, because of the 5:1 ratio of oxidant required, the molar ratio of formic acid to periodic acid is only 1:40 for a 10% glycerol solution, while in the second it is 1:4. To test this theory excess ethylene glycol was added to the reaction mixture at the end of the normal oxidation period and the unused periodic acid completely destroyed in a period of 20 minutes. This treatment was found completely to overcome the previous difficulty. By using this technique, all samples and blanks could be titrated to pH 7.6, allowing the use of Brom-thymol blue indicator where this is preferable to the continuous pH-meter. Fig. 4 shows the similarity of some of the curves when plotted and Table II shows the values obtained on a wide variety of concentrations of glycerol in the presence of other oxidizable material. It may be noted that where the

TABLE II

Analyses of Pure and Mixed Glycerol Solutions

Sample No.	Composition	Gr. Glycerol in Sample	% Glycerol in Sample	Ml. Titration (0.1N KOH)	Gr. Glycerol Found	% Glycerol Found	% Error
1	Pure glycerol	0.2119	100.00	22.40	0.2120	100.05	+0.05
2	Pure glycerol	0.1060	100.00	11.17	0.1056	99.62	—0.38
3	Pure glycerol	0.05319	100.00	5.62	0.05298	99.61	—0.39
4	Pure glycerol	0.02120	100.00	2.24	0.02119	99.95	—0.05
5	Glycerol + 0.5250 gr. diglycerol	0.01739	3.21	1.80	0.01704	3.14	—2.18
6	Glycerol + 0.5250 gr. diglycerol	0.03478	6.21	3.68	0.03484	6.22	+0.16
7	Glycerol + 0.1875 gr. diglycerol	0.02269	10.77	2.47	0.02330	11.05	+2.53
8	Glycerol + 0.3750 gr. diglycerol	0.06956	15.65	7.35	0.06958	15.65	0.00
9	Glycerol + 0.1875 gr. diglycerol	0.04388	18.96	4.54	0.04297	18.57	—2.06
10	Glycerol + 0.1875 gr. diglycerol	0.1075	36.44	11.32	0.1071	36.31	—0.36
11	Glycerol + 0.0750 gr. diglycerol	0.1739	69.87	18.32	0.1734	69.67	—0.29
12	Glycerol + 0.0375 gr. diglycerol	0.1739	82.26	18.37	0.1739	82.26	0.00
13	Glycerol + 0.0750 gr. diglycerol + 1 gr. NaCl	0.1739	69.87	18.34	0.1736	69.75	—0.17
14	Glycerol + 0.0750 gr. diglycerol + 5 gr. NaCl	0.1739	69.87	18.32	0.1734	69.67	—0.29
							Av.= ±0.64

TABLE IV

Analysis of Some Commercial Diglycerol Products

Sample No.	By Vacuum Distillation (0.2 mm.)[1]			By Periodic Acid Method[1]			Unit Deviation of the Methods		
	% Glycerol	% Diglycerol	% Polyglycerol	% Glycerol	% Diglycerol	% Polyglycerol	Glycerol	Diglycerol	Polyglycerol
1	1.5	80.9	18.4	0.70	80.9	18.4	−0.8	0.0	0.0
2	2.9	72.4	24.0	2.15	73.3	24.5	−0.7	+0.9	+0.5
3	3.0	76.3	20.6	2.31	75.1	22.6	−0.7	−1.2	+2.0
4	2.0	77.6	20.0	2.28	77.6	20.1	+0.3	0.0	+0.1
5	2.0	79.5	19.1	1.44	80.5	18.1	−0.6	+1.0	−1.0
6	1.5	76.7	21.6	2.67	76.1	21.2	+1.2	−0.6	−0.4
7	1.0	80.9	18.7	1.45	80.8	17.7	+0.5	−0.1	−1.0
8	4.0	76.9	20.0	3.16	74.5	22.3	−0.8	−2.4	+2.3
9	12.1	75.0	13.9	11.72	75.3	13.0	−0.4	+0.3	−0.9
10	4.8	76.0	18.8	4.54	76.0	19.5	−0.3	0.0	+1.3
11	10.5	73.3	15.0	9.52	73.5	17.0	−1.0	+0.2	+2.0
12	3.2	78.4	18.7	2.87	74.7	22.4	−0.3	−3.7	+3.7
13	2.9	80.3	17.3	2.45	84.1	13.4	−0.4	+3.8	−3.9
							Av. ±0.6	Av. ±1.1	Av. ±1.5

[1] On anhydrous basis.

glycerol is a very small fraction the error of the determination is slightly larger. This is undoubtedly due to the errors involved in estimating tenths of milliequivalents of organic acids in extremely dilute solutions. This can be partially overcome by using the exact sample weights given in Table III. In all cases use of these sample weights will give maximum accuracy.

TABLE III

Approximate Size of Sample Used for Analysis

% Glycerol Present	Sample to be Weighed in Grams	Sample to be Oxidized in 50 ml. Aliquots (gr.)
0-10	5.6-4.8	0.56-0.48
10-25	4.8-4.2	0.48-0.42
25-50	4.2-3.5	0.42-0.35
50-75	3.5-2.8	0.35-0.28
75-100	2.8-2.0	0.28-0.20

It has been previously reported (3) that sodium chloride interferes with the determination of glycerol by the usual periodate oxidation procedure. In the method here described it is without effect even when present in a 25:1 ratio as shown in examples 11, 13, and 14 in Table II.

Method

Reagents Required

Oxidants:

(a) Periodic acid—H_5IO_6 (for total oxidation value)—11 gr./liter—dissolved in 200 ml. distilled water and then 800 ml. glacial acetic acid added.

(b) Periodic acid—(for glycerol determination)—35 gr./liter in distilled water. Store both reagents in glass-stoppered amber bottles.

0.1N potassium hydroxide, aqueous solution.

0.2N sodium thiosulphate solution, standardized against potassium dichromate.

25% Potassium iodide solution.

50% Ethylene glycol (CP) in distilled water.

Brom-thymol blue indicator—0.1% in water.

Starch indicator solution.

Apparatus Required

Beckman pH meter (continuous recording).

100-ml. chamber burette—graduated in tenths of a ml.

5-, 10-, 20-, 25-, 50-ml. pipettes.

500-ml. Erlenmeyer iodine flasks.

600-ml. beakers.

500-ml. volumetric flask.

Procedure

I. *Determination of Per Cent Free Glycerol*

It is essential for this determination that the optimum sample is oxidized in order to give maximum accuracy. In order to arrive at this, Table III has been devised. A preliminary sample, assumed to contain around 100% glycerol, is oxidized (0.20-0.22 gr.) according to the following scheme and the approximate per cent glycerol calculated from equation (1). From this initial determination the correct sample weight is taken from Table III.

The appropriate sized sample is accurately weighed by difference into a 500-ml. volumetric flask, the sam-

Calculations

(1) $$\frac{(\text{ml. KOH sample} - \text{ml. KOH blank}) \times \text{N of KOH} \times 0.09209 \times 100}{\text{gr. sample in aliquot}} = \%\ \text{glycerol on ''as is'' basis.}$$

(2) $$\frac{(\text{ml. } Na_2S_2O_3 \text{ blank} - \text{ml. } Na_2S_2O_3 \text{ sample}) \times 23.02 \times \text{N } Na_2S_2O_3}{\text{gr. sample in aliquot} \times 10} = \text{''Total oxidizable as \% glycerol'' on ''as is'' basis.}$$

(3) $$\frac{\text{ml. } Na_2S_2O_3 \text{ sample}}{\text{ml. } Na_2S_2O_3 \text{ blank}} \times 100 = 78\% \text{ or greater.}$$

(4) $$\text{''Total oxidizable as \% glycerol'' or \% free glycerol} \times \frac{100}{100 - \%\ H_2O} = \text{corrected value on anhydrous basis}$$

(5) (a) % Glycerol (corrected) + 0.4523 × % diglycerol + 0.2900 [100 − % diglycerol − % glycerol (corrected)] = ''Total oxidizable as % glycerol'' (corrected).

or

(b) $$\frac{[\text{''Total oxidizable as \% glycerol (corrected)''}] - [0.7100 \times \%\ \text{glycerol (corrected)}] - 29.00}{0.1623} = \%\ \text{diglycerol on anhydrous basis.}$$

(6) 100 − [% diglycerol (corrected) + % glycerol (corrected)] = % polyglycerol on anhydrous basis.

(7) $$\%\ \text{diglycerol or \% polyglycerol on anhydrous basis} \times \frac{100 - \%\ H_2O}{100} = \text{values on ''as is'' basis.}$$

ple dissolved and made up to volume with distilled water. The pH of this solution must be checked to pH 6.2 (methyl red indicator) and any samples taken for analysis must be brought to this pH, by the addition of the necessary 1/10 normal acid or alkali in order not to interfere with the formic acid determination.

A pipetted 50-ml. aliquot of the solution to be oxidized is mixed with exactly 50 mls. of oxidant (b) in a 600-ml. beaker. The covered beaker is stirred by swirling and allowed to stand for one hour. At the end of this time 5 mls. of 50% ethylene glycol-water solution is pipetted in, mixed, and allowed to stand for an additional 20 minutes. Dilute the reaction mixture to 300 mls. and titrate with 0.1N KOH using the 100-ml. chamber burette to pH 7.60, employing a pH-meter or 12 drops Brom-thymol blue indicator. Stir continuously and add alkali slowly toward the end-point. Record the volume of KOH used to the nearest 0.01 ml. (The Brom-thymol indicator end-point is a sharp green to blue transition.)

Run a blank similarly with 50 mls. of water and 50 ml. of oxidant (b) and titrate in the same manner. Calculate the per cent of glycerol as given in equation (1) under calculations.

II. *Determination of "Total Oxidizable as % Glycerol" Value*

Pipette a 25-ml. aliquot of the solution used for the determination of free glycerol into a 500-ml. Erlenmeyer iodine flask. Pipette in 50 mls. of oxidant (a). Stir by gentle swirling and let stand for one hour. After the reaction period is complete add 18-20 mls. of 25% KI solution and titrate the liberated iodine with 0.2N sodium thiosulphate to a starch-iodine end-point, employing a 100-ml. chamber burette. Record this reading to the nearest 0.01 ml.

Run a blank and record the value. The difference between the sample and blank titrations must indicate the necessary excess for complete oxidation (equation 3). Where this difference indicates less than the 5:1 ratio required, a smaller aliquot or sample must be taken.

Equation (2) gives the "total oxidizable value as % glycerol."

III. *Determination of Water Content*

The water content of the sample should be known so that corrections necessary in equation (4) can be calculated. The Karl Fischer method for water content is very well suited. Using the appropriate sized sample determine water according to method in reference (7).

Results and Comments

The proposed method described has been used to analyze a large number of commercial samples of "diglycerol" and the data is shown in Table IV. Careful Claisen vacuum distillations of the same samples are compared with this new method.

The glycerine analysis described herein has certain unique advantages compared with other methods and is simple and easy to use. The application of the glycerine method to different problems such as glycerine crudes, etc., where large amounts of various impurities are present, may be feasible.

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Pilot-Plant Manufacture of Peanut Protein[1]

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MANY industrial uses of peanut protein and meal have been reported by this Laboratory in the fields of synthetic fibers (9) and adhesives (1, 3, 4, 5, 6). These specialized industrial uses for peanut protein require proteins such as those found in solvent-extracted meals, which are more soluble and less modified than those isolated from hydraulic-press or screw-press meals (5). This is one of the reasons that the development of the solvent-extraction process for the removal of the oil from peanuts is a prerequisite to the commercial development of peanut meal and protein as industrial raw materials. A process for the isolation of peanut protein from solvent-extracted meals has been previously reported (2). An improved process is reported in this paper in which the yield of protein has been greatly increased and operations simplified.

[1] Presented at the 39th Annual Meeting of the American Oil Chemists' Society, New Orleans, Louisiana, May 4 to May 6, 1948.
[2] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

Production of Solvent Extracted Meal

Peanuts were dried at 140° to 150°F. until the skins could be removed by aeration. The peanut kernels were flaked, and the oil was extracted in a batch extractor by means of n-hexane at room temperature as described in a previous publication (10). The bulk of the solvent was removed by aeration at room temperature, and the meal was further dried at 125° to 130°F. The dried meal contained about 1% lipids and 10% nitrogen.

Protein Manufacture

An outline of the various steps required in processing peanut meal for protein and by-products is shown in Fig. 1. Peanut meal is suspended in water (80 parts per million hardness and 150 parts per million solids) in the weight ratio of 10 parts of water to 1 part of meal and wetted by agitation in the protein peptizing tank (Fig. 2). Sodium hydroxide (aqueous, 30%) is added to the meal suspension