b) *Measurement of Thermal Expansion*

1. Immerse the dilatometer to the 300 mark in the 60°C. bath and record reading after 15 min. Rechecks of the 60°C. reading at the end of the determination should agree with the 60°C. reference reading. Significant variations indicate faulty technique.
2. Transfer the dilatometer to the 37.8°C. bath, and immerse to the 300 mark. Read level of indicator at intervals of 5 min. until the change is less than 2 units in 5 min. Record the readings.

   Note. It is necessary for the sample to be completely melted at the lower temperature. If any seeding or clouding of the sample occurs, the sample must be remelted in the 60°C. bath, and the temperature of the other bath must be raised. If the reference bath temperatures are changed, appropriate substitution must be made in the calculations.

c) *Conditioning of the Sample*

1. Transfer the dilatometer to the 0°C. bath, and immerse to the 300 mark and hold for 15 min.
2. Transfer to a 26.7°C. bath, and hold for 30 min.
3. Transfer back to 0°C. bath, and hold for 15 min.

   Note. If an ice bath is used, provisions should be made for adequate water-circulation.

d) *Measurement of Dilation*

1. Transfer the dilatometer from the 0°C. bath to a bath at the lowest desired temperature. Immerse to the 300 mark, and record reading at 30 min.
2. Repeat at the next highest temperature and so on until readings have been obtained at all of the desired temperatures.

E. CALCULATIONS

1. Solid fat index at temperature T is
   (total dilation) − (thermal expansion) × (60 − T)
   where
   T is observed temperature
   Vc(T) is volume correction for expansion of glass and water at T
   R(T) is dilatometer reading at T
   W is weight of sample.
2. Thermal expansion of sample per degree C in ml./kg. is

$$\frac{R(60) - R(37.8) - Vc\ (37.8)}{W \times (60 - 37.8)}$$

   (See Notes 3 and 4)

3. Total dilation between T and 60 C. in ml./kg. is

$$\frac{R(60) - R(T) - Vc(T)}{W}$$

VOLUME CORRECTIONS (Vc)

Bath temp. °C.	60°C. Reading				
	1,000	1,100	1,200	1,300	1,400
0	22.0	20.3	18.6	16.9	15.2
5	22.2	20.5	18.7	17.0	15.3
10	21.8	20.1	18.4	16.7	15.1
15	21.0	19.5	17.8	16.2	14.6
20	19.8	18.4	16.8	15.3	13.8
25	18.4	17.0	15.6	14.1	12.7
30	16.6	15.3	14.0	12.7	11.4
35	14.4	13.3	12.2	11.1	10.0
40	12.0	11.0	10.2	9.2	8.3
45	9.4	8.7	8.0	7.2	6.5
50	6.6	6.1	5.6	5.1	4.5
55	3.2	3.0	2.8	2.5	2.3
60	0	0	0	0	0

F. REPRODUCIBILITY. Collaborative studies have shown that the following reproducibility can be expected:

1. two single determinations made on different days by an analyst should not differ by more than approximately 2.8% of the value;
2. separate determinations by two different analysts in a laboratory should not differ by more than approximately 3.4% of the value; and
3. separate determinations in two different laboratories should not differ by more than approximately 4.1% of the value.

G. NOTES

1. The basic procedure described above is applicable at temperatures other than those specified, and the committee recognizes that sometimes such deviations are necessary. These depend on the composition and the character of the fat. It is hoped however that within limits a uniform temperature range may become established in the industry. Meanwhile further work is planned in this direction.
2. In order to meet the specifications of this method, the dilatometer scale must be accurate to 0.005 ml. or less (1 scale graduation) from 0 to 1,400. It is necessary to draw correction curves from the calibration data for those dilatometers which do not meet specifications, and corrected readings must be used to calculate the solid fat index.
3. Vc from the table represents the combined corrections for the expansion of glass and water and applies to Pyrex glass only. If dilatometer is constructed of glass other than Pyrex, the corrections must be redetermined.
4. The normal liquid thermal expansion is 0.83 − 0.85 ml./kg. If determined values differ from this, it is advisable that they be rechecked carefully.

Report of the F.A.C. Total Neutral Oil Subcommittee 1956-1957

THE TOTAL NEUTRAL OIL SUBCOMMITTEE of the Fat Analysis Committee of the American Oil Chemists' Society was appointed in 1953 to select a standard method for the determination of total neutral oil.

Three methods were considered for study by the subcommittee: a modification of the Wesson method, J. Oil and Fat Industries, *3,* 297–305 (1926); modifications of the chromatographic method as proposed by Linteris and Handschumaker, J. Am. Oil Chemists' Soc., *27,* 260–264 (1950), and the crude oil impurities technique, which is an estimate based on the summation of the acetone-insoluble, free fatty acids, and moisture content of the sample. The latter technique was discarded as a possible method because it was not a single procedure. The chromatographic and Wesson techniques were studied quite extensively by the subcommittee.

In 1954 a sample of crude cottonseed oil was analyzed by the subcommittee, using the Wesson method and the chromatographic method. Each collaborator ran the chromatographic method, using the same alumina as well as his own supply of alumina. The statistical analysis of the 1954 study indicated that the precision of the Wesson method and the chromatographic method was comparable and that the agreement among laboratories using their own alumina for the chromatographic method was satisfactory.

In 1955 a "nested design" was used by the subcommittee to compare the Wesson method with the chromatographic method proposed by Archer-Daniels-Midland, using six different crude oils. The statistical analysis of the 1955 study indicated that the precision of the chromatographic method was as good as, if not superior to, the Wesson method. Since the majority of the subcommittee members favored the

chromatographic procedure, it was decided to confine further studies to this technique.

Because it is desirable to have as simple a technique as possible for an official A.O.C.S. method, the less complex 1954 chromatographic technique, revised to contain certain features of the Archer-Daniels-Midland method, was compared with the Archer-Daniels-Midland method in the 1956 study. Again the subcommittee utilized the "nested design" technique to evaluate the two chromatographic methods, using three different crude oil samples. The statistical analysis of the 1956 study showed that the 1954 method as revised gave the highest degree of precision both within and among laboratories.

The subcommittee recommends that the 1954 method, as revised, be adopted by the Society as a tentative method for total neutral oil.

The statistical analyses were made by H. P. Andrews, head of the Statistics Division, Swift and Company Research Laboratories, Chicago, Ill.

R. J. BELL A. F. KINGSLEY
R. A. DECKER W. A. PONS JR.
K. E. HOLT S. E. TIERNEY, chairman

Collaborative Study of Total Neutral Oil Methods, 1954 Statistical Analysis

Total neutral oil determinations were made by seven laboratories on a collaborative sample of crude cottonseed oil, using the Wesson method, the chromatographic method with alumina provided, and the chromatographic method with their own alumina. Most determinations were made in triplicate (a few in duplicate). The statistics are given in Table I.

It is apparent from the tabulated means that the Wesson method gave consistently higher values.

The within-laboratory variances show that the ability to repeat determinations was quite comparable for the Wesson method and the chromatographic, using the alumina provided. When using their own alumina for the chromatographic method, the precision was not as good.

The among-laboratory variances show that the laboratories agree more closely on the chromatographic method, using the alumina provided, than for the other two. It should be pointed out however that if the extremely low values reported by Collaborator No. 3 for the chromatographic method, using own alumina, had not been included, the agreement would have been quite comparable to the chromatographic, using the alumina provided.

Numbers in the brackets preceding the means are the relative positions within the method. From this it can be seen generally that if a company reported among the highest by one method, this was true also for the other two.

Collaborative Study of Total Neutral Oil Methods, 1955 Statistical Analysis

In the collaborative study of total neutral oil methods, five laboratories (Swift, Southern Utlization Research Branch, Spencer Kellogg, Anderson Clayton, and Archer-Daniels-Midland) made analyses of six different oils, using the ADM Chromatographic method and the Wesson method. The standard design for collaborative studies was used. In each laboratory, analyses were made by two different analysts on two different days in duplicate.

The data were subjected to statistical analysis to isolate the components of variance associated with the different sources of error. The means for the different laboratories on the different oils are summarized in Table VI.

The variance analyses were computed separately for each of the five different samples, but since the estimates were all of similar magnitude, they were pooled together for all of the oils. Those variance components are tabulated in Table II.

The above estimates were made by using the entire data. A relatively large amount of the variability was found to come from within laboratories rather than among laboratories. For this reason the variance components were computed for each laboratory, and these are summarized in Table III.

The asterisk (*) indicates that the within-laboratory variation was excessive in Collaborator 4's laboratory, and the data revealed that most of that variation was in the results of one of the analysts. The committee member from that company has cited this fact and suggested that the results of that analyst probably should be omitted from the interpretation. When such an omission was made, the variance components for the other analyst (Table IV) were in very much better agreement with those in the other companies.

Having adjusted the statistical analyses for those discrepant results, it is possible to make a more valid comparison of the two methods. Since only three of the companies ran the samples by both methods, the variances from those are summarized in Table V.

TABLE I
Means

Collaborator No.	Wesson method	Chromatographic method	
		Alumina provided	Own alumina
1	(6) 97.383	(3) 97.420	—
2	(7) 97.360	(7) 97.020	(5) 97.107
3	(5) 97.420	(4) 97.285	(6) 96.475
4	(1) 98.313	(5) 97.220	(4) 97.353
5	(3) 97.925	(2) 97.490	(2) 97.510
6	(2) 98.005	(1) 97.800	(1) 97.745
7	(4) 97.597	(6) 97.213	(3) 97.400
Mean value for method	97.703	97.321	97.284
Variances (S^2)			
Among laboratories	0.1386	0.0532	0.1424
Within laboratories	0.0191	0.0107	0.0544
Among lab. means	0.1461	0.0574	0.1642

TABLE II

	Chromatographic	Wesson
Among laboratories	.0824	.0266
Within laboratories		
Analysts	.0231	.0600
Days	.1099	.0293
Duplicates	.0448	.0524
Total	.1778	.1417
Total	.2602	.1683
Standard deviation	0.51	0.41

TABLE III

Collaborator No.	Chromatographic				Wesson			
	Analysts	Days	Duplicates	Total	Analysts	Days	Duplicates	Total
1	.0686	.0133	.0221	.1040	.0645	—	.0511	.1156
2	—	.0024	.0017	.0041	.1083	.0226	.0184	.1493
3	—	.0079	.0222	.0301				
4	—	.5529	.1717	.7246*	.0632	—	.1135	.1767
5	.0022	—	.0016	.0038				
Average				.1778				.1417

TABLE IV

Collaborator No.	Chromatographic			Wesson		
	Days	Duplicates	Within anal.	Days	Duplicates	Within anal.
4	.1150	.0803	.1853	—	.0157	.0157

TABLE V

Collaborator No.	Chromatographic				Wesson			
	Analysts	Days	Duplicates	Total	Analysts	Days	Duplicates	Total
1	.0686	.0133	.0221	.1040	.0645	—	.0511	.1156
2	—	.0024	.0017	.0041	.1083	.0226	.0184	.1493
4	—	.1150	.0703	.1853	.0632	—	.0157	.0789
Average				.0978				.1146
Standard deviation				0.31				0.34

From the standpoint of repeatability within a laboratory, the two methods were essentially equal, and individual analyses by different analysts within a laboratory should not differ by more than 0.90 with either method.

Concerning the tabulation of the mean values for the various laboratories, it was observed that the Chromatographic method gave slightly lower total neutral oil values than did the Wesson method on all the oils except coconut. On coconut oil the Chromatographic method gave slightly higher total neutral oil values than the Wesson method. This trend was observed consistently in all of the laboratories.

TABLE VI

Collaborative Study of Total Neutral Oil Methods, 1955
Summary of Means

Collaborator No.	Chromatographic method						Wesson method					
	Hy. C/S	Exp. C/S	Ex. S/B	Peanut	Coconut	Linseed	Hy. C/S	Exp. C/S	Ex. S/B	Peanut	Coconut	Linseed
1	96.16	96.06	98.29	95.87	93.10	97.01	97.25	97.18	98.90	96.31	92.88	97.85
2	96.79	96.71	98.75	96.61	94.15	97.37	96.73	96.81	98.93	96.11	92.66	97.12
3	96.45	96.25	98.56	96.23	93.63	96.99	—	—	—	—	—	—
4	95.95	95.92	98.21	95.88	93.30	96.94	96.81	97.13	99.08	96.33	92.90	97.52
5	96.95	96.83	98.83	96.52	93.98	97.29	—	—	—	—	—	—
Average	96.46	96.35	98.52	96.22	93.63	97.12	96.93	97.04	98.97	96.25	92.81	97.50

Collaborative Study of Total Neutral Oil Methods, 1956 Statistical Analysis

In the 1956 Total Neutral Oil study the modified 1954 Chromatographic method was compared with the Chromatographic method of Archer-Daniels-Midland. Five laboratories (Archer-Daniels-Midland, Southern Utilization Research Branch, Spencer Kellogg, Swift, and Anderson Clayton) made duplicate analyses, on each of two days by each of two analysts, of three different oil samples (cottonseed solvent, cottonseed expeller, and soybean solvent).

The data were subjected to statistical analysis to isolate the components of variance associated with the different sources of error. The means for the different laboratories' results on the different oils are summarized in Table IX.

Collaborator No. 5's analysts used cotton plugs in the 1954 modification method instead of the prescribed fritted disc and, since a cursory examination of the data revealed consistently low results with that method, that laboratory's results were omitted from the variance calculations.

The resultant variance components are tabulated in Table VII along with those obtained in the similar 1955 collaborative study.

With all these studies the major component of variance has been within the laboratories, and of that within-laboratory variance the day-to-day component was the largest and variation between analysts was the smallest component in each instance. As indices of relative variation within the different laboratories, the individual components have been tabulated in Table VIII.

TABLE VII

	1955 Study	1956 Study	
	ADM	ADM 1956	1954 Modification
Among-labs. variance	.0955	.0142	.0003
Within-labs. variance	.0824		
Analysts	.0229	—	—
Days	.0436	.0357	.0309
Duplicates	.0313	.0075	.0025
Total	.0978	.0432	.0334
Total variance	.1802	.0574	.0337
Standard deviation (single analysis)	0.42	0.24	0.18

TABLE VIII

Collaborator No.	ADM (1956)				1954 Modification			
	Duplicates	Days	Analysts	Total	Duplicates	Days	Analysts	Total
1	.0065	.0074	—	.0139	.0035	.0025	.0107	.0167
2	.0005	.0007	.0115	.0127	.0035	.0038	.0085	.0158
3	.0022	.0287	—	.0309	.0017	.0188	.0070	.0275
4	.0129	.1331	—	.1460	.0014	.0804	—	.0818
5	.0152	.0011	—	.0163	.0160	—	.0032	.0192

From the components in Table VII it is apparent that the 1954 modification was the method having the highest degree of precision both within and between laboratories.

Some statistical limits for differences (5% probability), which may be of specific interest, are tabulated below:

	ADM 1956	1954 Modification
Between duplicates (same day and analyst)	0.24	0.14
Between single analyses on different days	0.59	0.52
Between single analyses from different laboratories	0.68	0.52

TABLE IX

Collaborative Study of Total Neutral Oil Methods, 1956
Summary of Means

Collaborator No.	ADM—1956			1954 Modification[a]		
	C/S Solvent	S/B Solvent	C/S Expeller	C/S Solvent	S/B Solvent	C/S Expeller
1	96.25	98.63	97.98	96.27	98.58	98.15
2	96.40	98.74	98.26	96.46	98.76	98.35
3	96.46	98.72	98.24	96.35	98.60	98.26
4	96.31	98.60	97.89	96.26	98.68	98.15
5	96.50	98.79	97.71	95.82	98.15	97.16
Average	96.38	98.70	98.02	96.34	98.65	98.23

[a] Collaborator No. 5's results, with this method, not used in calculating averages.

Neutral Oil

Definition. The total neutral oil of natural fats and oils, consisting essentially of triglycerides and unsaponifiable matter, is determined by this method. The free fatty acids and miscellaneous non-fat substances are removed by passing through a column of activated alumina.

Scope. This method has been satisfactorily applied by the committee to cottonseed, soybean, peanut, linseed, and coconut oils. Application of this method to other oils has not been investigated by the committee, but it is probably applicable to practically all natural animal and vegetable fats and oils.

A. Apparatus

Chromatographic tubes, 20 mm. in diameter x 400 ml. in length with sealed-in coarse porosity fritted disc, Corning Glass Works Cat. No. 38,450 or equivalent.

Beakers, 150-ml., 250-ml., and 400-ml., and 1-liter.
Soxhlet flask, 250-ml.

Funnel, powder-filling type. The following dimensions are convenient:
diameter of top, 65 mm.
length of stem, 25 mm.
outside diameter of stem, 14 mm.

Desiccator containing an efficient desiccant. Calcium chloride is not satisfactory. (See A.O.C.S. Specification H 9-45.)

B. Reagents

Ether-methanol solvent, prepared by mixing 25 ml. of methanol (A.C.S. grade) with 975 ml. of absolute ethyl ether (A.C.S. grade).

Aluminum oxide—activated alumina grade F-20, Mesh 80-200 (Aluminum Ore Company, East St. Louis, Ill., or equivalent).

(Note: The alumina must be kept free from moisture at all times. This can be accomplished by transferring the alumina as received to 2-oz. jars and storing in a desiccator until ready for use.)

C. Preparation of Sample

The sample container must be vigorously shaken and the sample thoroughly mixed in order to incorporate and uniformly to distribute meal or other sediment. If the oil is cold, heat to 20°C. (50°C. for soybean oil and 38°C. until completely melted for coconut oil) before shaking. Inspect the inside of the container to be sure that no sediment remains clinging to the sides or bottom. If any sediment is found, remove it completely (cut the can open if necessary) and incorporate thoroughly with the oil.

The uniform incorporation and distribution of settlings and suspended matter are very significant in determining the accuracy of the result of the analysis. If the results are to be expressed on the basis of oil only, *i.e.*, exclusive of water and foreign material, these should be removed from the portion to be analyzed by filtration through a clean, dry filter paper before weighing.

D. Preparation of the Column

Attach a short piece of rubber tubing equipped with a pinch clamp to the bottom of the chromatographic tube. Fill the tube about one-third full with the ether-methanol solution. Open until about 5 ml. drain from the tube and no air is trapped in the bottom of the tube; then close. Weigh 20 ± 1 g. of activated alumina and transfer into the tube with the aid of a powder funnel. Wash down any alumina remaining on the wall of the tube with a few ml. of solvent.

E. Procedure

Weigh a sample of appropriate size, depending upon the anticipated neutral oil content, into a clean and dry 100-ml. beaker.

Approximate neutral oil	Weight of sample
100–90	2–3 ± 0.001 g.
90–75	1–2 ± 0.001 g.
75–50	0.7–1 ± 0.001 g.
50–0	0.45–0.55 ± 0.001 g.

Add 25 ml. of the ether-methanol solution and swirl to dissolve the sample. Just before pouring the sample solution on the column, remove the rubber tubing at the bottom and allow the excess solvent to drain until the level of the solution is 5 mm. above the level of the activated alumina. Immediately add the sample-solution by pouring the contents on the column, being careful not to disturb the surface of the alumina.

Collect the percolate in a previously dried and tared 250-ml. beaker or Soxhlet flask. Use a total of 25 ml. of ether-methanol solution, divided into four equal portions, to effect the transfer of the sample to the column, adding each washing after the preceding one is only 5 mm. above the top of the alumina.

When the last wash has gone into the alumina except for the 5 mm. remaining above the column, add 100 ml. of ether-methanol solution. Continue collecting the percolate until all the ether-methanol has passed through the column. Wash the drawn end of the tube with a small portion of ether-methanol solution and add to the 250-ml. beaker.

Evaporate the ether-methanol solution on a water bath with the aid of a gentle stream of air. After the solvent fumes have disappeared, remove from the steam bath and place in 105°C. oven for one hour. Remove from the oven, cool in a desiccator, and weigh the beaker and contents.

F. Calculations

$$\text{Neutral oil content, \%} = \frac{100\ (\text{weight of residue})}{\text{weight of sample}}$$

G. Reproducibility

Collaborative studies have shown that the following reproducibility can be expected:

Duplicate determinations made on the same day by an analyst should not differ by more than approximately 0.14.

Averages of duplicate determinations made in two different laboratories should not differ by more than approximately 0.37.

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Absence of Thermal Polymers in Potato-Chip Frying Oils[1]

DANIEL MELNICK, Research Laboratories, The Best Foods Inc., Bayonne, New Jersey

DURING the past year there have been articles and statements published in both the lay and scientific press on the disadvantages of fat in the diet. These published statements have neglected the many scientific reports dealing with the noncaloric, essential functions of fats in the diet (1) and particularly the importance therein of certain polyunsaturated essential fatty acids (2). In these general attacks on all types of fats in the diet there is one type of fat regarded by many to be at the bottom of the scale of foods acceptable for human consumption; this is the fat absorbed in fried foods. Toxic polymers have been alleged to be formed during commercial frying operations, and questions have been raised about the possibility of fatty acid isomers developing in these operations.

Publications on the harmlessness of the fats absorbed by fried foods are unfortunately scanty in number. It is the purpose of the present report to review critically what has been published on this subject and to describe the rationale in support and the results of a nation-wide survey of the potato chip industry to determine the extent of polymer formation in the frying oils and the nutritional significance of the findings.

Potential Thermal Polymers in Frying Oils Employed by the Potato Chip Industry. Ease of polymer formation is directly related to the degree of unsaturation of the fatty acids (3). Likewise during hydrogenation of an oil there is a preferential uptake of hydrogen by the more highly unsaturated fatty acids. From the practical standpoint the present study need be concerned only with the possibility of dimers and higher polymers being formed from the linoleic acid in the frying oils. None of the oils employed by the potato chip industry contains linolenic acid. Unhydrogenated soybean oil contains about 8% of this fatty acid, but no potato chip manufacturer in this country uses in his operations such soybean oil because of flavor instability. Soybean oil shortenings contain no linolenic acid.

On heating linoleic acid for a period of time at a sufficiently high temperature, there occurs first a migration of the double bonds to a conjugated position. Such a linoleic acid isomer reacts with natural linoleic acid to form a dimer (4). As a result of this reaction there occurs a reduction in unsaturation from four double bonds to two double bonds. The

[1] Presented at the 20th Annual Conference, National Potato Chip Institute, Dallas, Tex., January 21, 1957.