TABLE VIII

Chemical and Physical Characteristics of Monohydric Esters–Tall Oil Fatty Acids

Ester	Boiling range °C. @ 2–4 mm.	Acid value	Sap'n value	Iodine value	Refractive index (20°C.)	Gardner color	
						Initial	Distillate
Methyl	156–160	3.0	187	118	1.4581	4	1
Butyl	182–188	3.2	163	105	1.4593	5	1
Amyl	187–190	1.7	160	99	1.4587	7	1
n-Pentyl	183–187	3.6	161	100	1.4590	10	1
n-Hexyl	185–189	2.6	155	94	1.4596	6	1
Iso-hexyl	216–223	2.5	155	94	1.4600	4	1
2-Ethylhexyl	224–230	2.6	142	90	1.4600	5	1
Iso-octyl	216–218	1.0	145	88	1.4600	6	1
n-Decyl	214–220	1.0	135	80	1.4620	7	1
Isodecyl	195–200	5.4	128	84	1.4634	5	2
Tridecyl	220–225	2.3	120	75	1.4639	7	2
Hexadecyl	243–248	1.0	112	69	1.4634	6	1
Eicosyl	.....	6.0	95	57	1.4663	6	..

sorbing gums formed during the reaction and heating and prevented the formation of insoluble resins in the still bottoms.

While the greater part of this work was done on the production of 2 ethylhexyl esters suitable for use in the preparation of epoxy plasticizer derivatives, it was also decided to prepare a series of tall oil esters of other commercially-available alcohols.

Various alcohol samples were solicited from Carbide and Carbon Chemicals Company, Enjay Company Inc., and Shell Chemical Corporation. A listing of these esters prepared from the alcohols by a variety of the above methods is presented in Table VIII. Esters of butyl and higher alcohols were made, using 1.1 mole of alcohol for each mole (280 g.) of fatty acid. Catalyst concentration was 0.3%.

The initial ester after a purification treatment of water washing, vacuum topping, and drying was distilled. Distillation was by batch method, using a vacuum of 2–4 mm. A small precut was removed, then the main portion of the ester, boiling over the distillation range as listed in Table VIII, was collected and used in subsequent analyses. The residue in all instances contained the major portion of color bodies. Initial colors are listed for the esters prior to distillation. All distillates were clear, water-white liquids.

While the above listings of esters and their properties is by no means complete, it does serve to illustrate the ease of esterification and variety of useful chemical entities which can be prepared from purified tall oil fatty acids, which are now available commercially.

REFERENCES

1. Christenson, A.M., and Harpt, R.E., U.S. Patent, 2,573,890 (1951).
2. Mayer, V., Jacini, C., and Jacini, G., Italian Patent, 467,994 (1951).
3. Freeman, S.E., and Glover, S.W., U.S. Patent, 2,423,232 (1947).
4. Markley, K.S., "Fatty Acids," Interscience Publishers Inc., New York, 1947, p. 268.
5. Eckey, E.W., J. Am. Oil Chemists' Soc., *33*, 575–579 (1956).
6. Konen, J.C., Clocker, E.T., and Cox, R.P., J. Am. Oil Chemists' Soc., *22*, 57–60 (1945).
7. Althouse, P.M., Hunter, G.W., and Triebold, H.O., J. Am. Oil Chemists' Soc., *24*, 257–259 (1947).

[Received April 7, 1960]

Analyses of Lipids and Oxidation Products by Partition Chromatography. Dimeric and Polymeric Products[1]

E.N. FRANKEL, C.D. EVANS, HELEN A. MOSER, D.G. McCONNELL, and J.C. COWAN,
Northern Regional Laboratory,[2] Peoria, Illinois

A liquid-partition chromatographic method was developed to determine dimers in fats. Silicic acid treated with 20% methanol in benzene served as the immobile phase. A mixture of 2% methanol in benzene was the mobile solvent. Chromatographic separation of free fatty acids from oxidized-deodorized oils gave three well-isolated fractions composed of unoxidized acids, dimeric or polymeric fatty acids, and polar fraction (ethyl ether eluate). Recovery of acidic materials from the column was essentially quantitative (96–100%), reproducibility was good, and the standard error of regression was ±0.26.

A linear relationship exists between the dimer content of deodorized soybean oil and the peroxide value of the oil before deodorization. An increase of 1% in dimer concentration corresponds to an increase in peroxide value of approximately 40. Dimer content of different vegetable oils varied from 1 to 3%.

The chromatographic method can be used to estimate the degree of oxidation that an oil has received before deodorization and to follow various phases of fat oxidation, polymerization, and processing.

THE EXTENT OF OXIDATION in deodorized vegetable oils is difficult to determine because deodorization rapidly destroys hydroperoxides. A quantitative measure of this "hidden oxidation" would help to predict the quality and future stability of an oil. Oxidation at different stages of processing and before deodorization is detrimental to stability of vegetable oils (1,6). This detrimental effect of oxidation in soybean oil is attributed to dimerization products derived from hydroperoxides by their decomposition prior to or during deodorization (2). The term "dimer" describes polymeric products derived from autoxidation and known to be principally dimeric in nature. The dimers were isolated from autoxidized-deodorized soybean oil and autoxidized-heated fatty esters and were characterized chemically and spectroscopically (3). They contain approximately 1 mole hydroxyl, 0.5 mole carbonyl, and 2 double bonds per mole of dimer. Attempts to show the presence of ether linkages and of a 6-membered ring in their structure failed.

The present paper deals with a liquid-partition chromatographic method used to determine dimers in oxidized-deodorized or heated fats. Since these dimers are derived from fatty acid hydroperoxides, this chromatographic method is useful in estimating the degree of oxidation that an oil has received before deodorization. This method also determines and characterizes dimers derived by thermal polymerization of conjugated or nonconjugated fatty acids and methyl esters. The method has been applied to determine hydroper-

[1] Presented at spring meeting, American Oil Chemists' Society, Dallas, Tex., April 4–6, 1960.
[2] This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture.

oxides in autoxidized fatty acids and fatty acid esters (4), to isolate hydroxy fatty acids and esters, and to analyze mixtures of mono-, di-, and triglycerides.

Experimental

Materials. Mallinckrodt's silicic acid, 100-mesh, labelled "suitable for chromatographic analysis by the method of Ramsey and Patterson," was the adsorbent. It was dried before use by heating over-night in a 1-in. layer at 120°C. Reagent-grade solvents were not purified further. The preparation of fatty esters, their oxidation, and polymerization were described previously (3). Dimers used as standards for chromatographic analyses came from molecular distillation of oxidized fatty esters, *i.e.*, hydroperoxides were polymerized at 210°C. in nitrogen for 15 min. Fatty acids and dimeric acids were prepared by saponification of oils or methyl esters, according to the A.O.C.S. Method Ca 6b-53, followed by extraction with ethyl ether to remove unsaponifiable material. Soap solutions were acidified to a pH below 2, and liberated acids were extracted with ether, washed with water, and dried over sodium sulfate.

Chromatography. The liquid-partition method was developed for free fatty acids because titration was comparatively easy and sensitive. Application of the method to methyl esters of fatty acids may also prove desirable for certain separations because the effect of hydroxyl and carbonyl groups is largely depressed when the dimers are chromatographed as free acids. The standard column was 24 x 400 mm. with a fritted-glass plate at the bottom and a 500-ml. reservoir on the top. The "immobile" solvent consisted of 20% methanol in benzene and the "mobile" solvent of 2.0% methanol in benzene (v/v). Immobile solvent (40 ml. in 5-ml. portions) and dry silicic acid (50 g.) were thoroughly mixed in a mortar with pestle until all lumps were completely broken. A volume of 100 ml. of mobile solvent was added with rapid mixing to slurry the adsorbent. The slurried mixture was poured in the column in two portions and packed with air pressure to a constant height, always maintaining a solvent head over the adsorbent. The sample (0.1–0.2 g.) of acids was dissolved in mobile solvent and applied to the column when the solvent head was a few millimeters above the adsorbent. The sample was mixed evenly into the top layer of adsorbent with a stirring rod. After using two 5-ml. portions of mobile solvent to rinse the top of the column, air pressure (5 to 10 p.s.i.) was applied until the sample was on the column. The mobile solvent (350 ml.) was added, and sufficient air pressure was applied to maintain an elution rate of 60 drops per minute. A collector was set to obtain 5-ml. fractions. In highly oxidized samples, ethyl ether was added to the column at the end of the run to elute a polar fraction. In this manner all material added to the column was completely recovered.

Analytical Methods. Chromatographic separation of the acids was followed by titration of the fractions with 0.2 N alcoholic potassium hydroxide (95% ethanol) to a thymol blue end-point. The solution was stirred by a gentle stream of nitrogen during titration with a Gilmont microburette. A more complete description of the method and equipment is given by Jones and Stolp (7). Molecular weights of fractions were determined cryoscopically in wet benzene (3). The organoleptic evaluations were made by a taste-panel procedure as described by H.A. Moser *et al.* (8).

Results and Discussion

Initially the chromatographic method was developed to separate fatty acid hydroperoxides from unoxidized fatty acids and esters (4). It was quickly observed that the dimeric acids derived from thermally-decomposed hydroperoxides had retention volumes similar to those of the corresponding fatty acid hydroperoxides. Consequently optimum conditions for separating fatty hydroperoxides were also optimum for separating their corresponding thermal dimeric acids from unoxidized material. Figure 1 shows the relationship between elution of dimer acids and solvent compositional changes in the eluate. In a run without a sample (dotted line) the concentration of methanol in the eluate remained constant at 0.25% for the first 250 ml. and then increased sharply to 1.3%. During the column development silicic acid absorbs methanol from the mobile phase until a new equilibrium is established. Elution of dimer acids (solid line) is coincident with the abrupt increase in the concentration of methanol in the eluate which, under these conditions, was from 0.25 to 1.30%. The separation between monomeric fatty acids and dimeric acids occurs when the concentration of methanol changes in the eluate. Column characteristics appear similar to those described by van Duin (10), who suggested simultaneous operation of liquid-liquid partition and liquid-liquid interface adsorption when the carrier is loaded with less than its maximum capacity for the immobile phase.

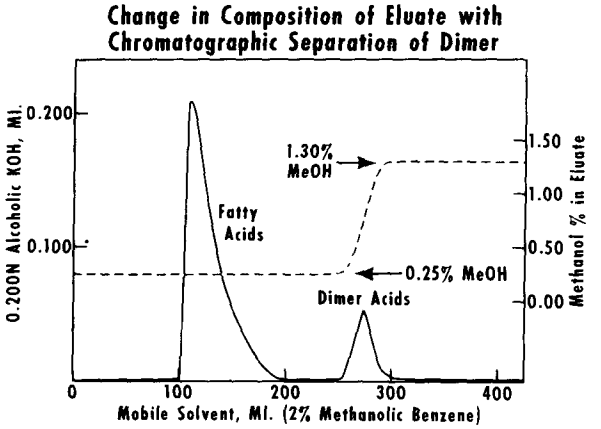

FIG. 1. Relationship between the change in composition of eluting solvent and the chromatographic separation of dimer acids.

Refined soybean oil was oxidized to different levels before deodorization. The oxidized oils were deodorized, then converted to acids by alkali saponification and ether extraction. Chromatographic separation of soybean fatty acids at four levels of oxidation is represented in Figure 2. Chromatographic fractions from the most oxidized sample were recovered and methylated with diazomethane for molecular-weight determinations. The first fraction was monomeric (M.W. 299), and the second was dimeric (M.W. 602). The third fraction, referred to as polar, had a molecular

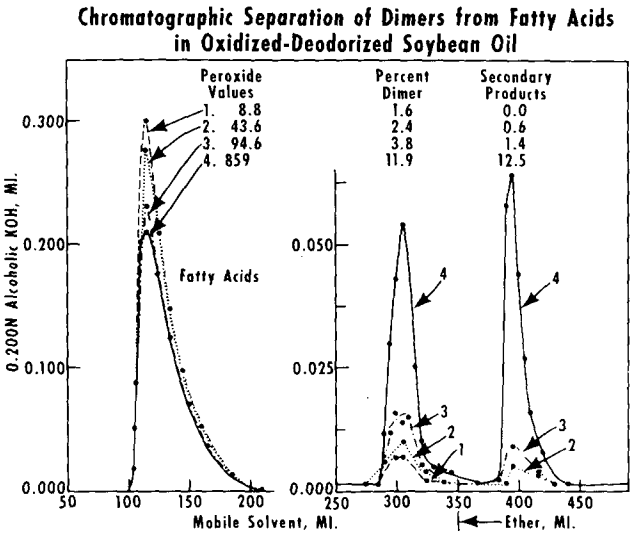

FIG. 2. Chromatographic separation of dimeric acids obtained by saponification of autoxidized-deodorized soybean oil. Oils oxidized to peroxide values of (1) 8.8, (2) 43.6, (3) 94.6, and (4) 859.

weight of 489, which indicated a complex mixture of polymeric and fragmented material.

Molecularly distilled dimeric acids (3), fractionated from oxidized-heated methyl oleate and methyl linoleate, are represented in Figure 3. The elution

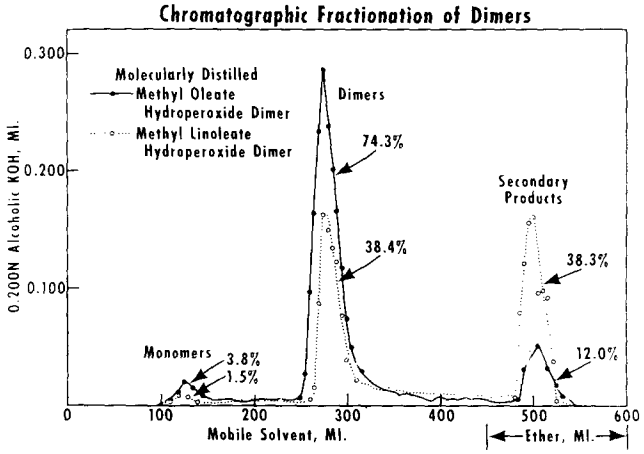

FIG. 3. Chromatographic separation of molecularly-distilled dimeric acids obtained by autoxidation of methyl oleate and methyl linoleate. Methyl oleate oxidized to a peroxide value of 1110 and methyl linoleate to 2210.

curves show the presence of a minor amount of monomer and of two polymeric fractions. The polar fraction was a major product in the polymer of oxidized methyl linoleate. The relative proportion of the polar fraction increased with the degree of oxidation. The formation of the polar fraction and its relationship to the peroxide level, the conditions of oxidation, or the type of substrate have not been completely investigated.

Table I shows good duplication of dimer analyses on soybean oil that were oxidized before deodorization at three widely different levels. These results, coupled with those shown in Figure 4, demonstrate that there is a direct relationship between the peroxide value

TABLE I

Duplicate Dimer Analysis of Deodorized Soybean Oil

Peroxide value before deodorization	Monomer	Dimer	Ether eluate	Total recovery
me./kg.	%	%	%	%
8.1	a) 95.7	1.7	.....	97.4
	b) 94.6	1.9	.....	96.3
130.0	a) 95.0	4.5	.....	99.5
	b) 93.0	5.1	.....	98.1
704.0	a) 57.7	15.2	.....	.....
	b) 57.9	16.9	21.4	96.2

before deodorization and the concentration of dimer after deodorization. The correlation coefficient between peroxide value and dimer content at these levels of oxidation was calculated to be 0.97. The standard deviation of the experimental points about the regression line was ±0.264. An increase of 1% in dimer concentration is equal to an increase in peroxide value of 43.5 me./kg. (Figure 4). A manometric de-

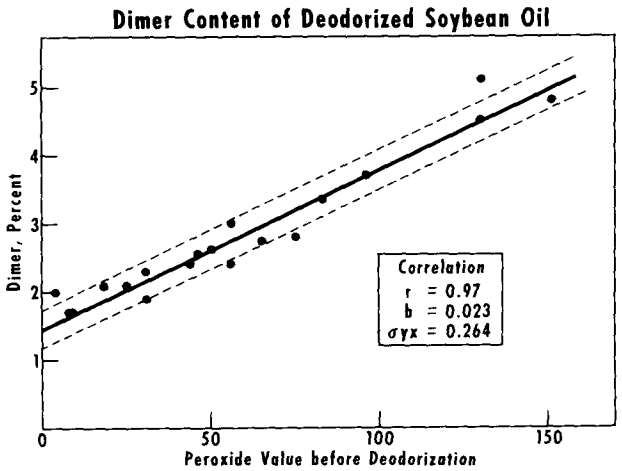

FIG. 4. Relationship of peroxide content of soybean oil before deodorization to the dimeric acid content after deodorization.

termination of oxygen absorption of soybean oil also showed good correlation between oxygen uptake and dimer formation (correlation coefficient = 0.97). The formation of 1% dimer corresponded to an oxygen uptake equivalent to a peroxide value of 64 and to an iodometric peroxide value of 51. Evidently the iodometric determination of peroxide value does not measure the total oxidation occurring in soybean oil. The quantity of polar fraction eluted with ether appears to be associated with the difference between iodometric peroxide value and oxygen uptake.

A linear relation was previously reported between carbonyl content and peroxide value in soybean oils before deodorization (2). The carbonyl method of Henick *et al.* (5), used in the earlier study, proved more sensitive than the present chromatographic method. The difficulty with most colorimetric carbonyl methods in the analysis of autoxidized fats is their inadequate standard, poor precision, and lack of agreement between methods. Since the chromatographic method determines dimers derived from fatty hydroperoxides, results can be directly related to given levels of oxidation in the oils.

A close relationship exists between the flavor score of aged samples, their dimer content, and the peroxide level of the oil before deodorization. The cor-

relation of dimer content to peroxide level was 0.99 for soybean oil A and 0.98 for the cottonseed oil (Table II). In cottonseed oil a peroxide level of 60 was required to produce a 1% increase in dimer content whereas two different lots of soybean oil required peroxide values of 41 and 37, respectively. Correlations between flavor scores and dimer contents are good when a given oil is oxidized to various peroxide levels. The correlation coefficient between score and dimer content in the aged samples was −0.83 for soybean oil A and −0.76 for the cottonseed oil. Data in Table II agree with those previously published (2) and also show that the quality of cottonseed oil, like that of soybean oil, decreases with increasing dimer contents.

TABLE II

Flavor Stability and Dimer Content of Oxidized Oils

Oils	Peroxide value before deodorization	Flavor score [a]		Dimer
		Initial	4-Day storage	
	me./kg.			%
Soybean oil A				
0-time	0.73	7.8	5.2	1.4
92 hours [b]	40.8	5.8	4.5	2.8
140	67.3	5.1	4.5	3.1
188	108.0	5.5	3.4	4.4
236	155.0	4.8	4.0	5.6
Soybean oil B				
0-time	8.1	8.0	6.4	1.7
146 hours	63.9	8.0	4.8	2.2
Soybean oil C				
0-time	15.3	6.5	5.7	1.4
120 hours	118.0	6.8	3.3	3.0
Cottonseed oil				
0-time	3.22	8.2	5.7	2.4
92 hours	47.9	5.8	5.4	3.4
140	72.0	6.2	5.6	4.2
188	107.0	6.1	5.2	4.4
236	136.0	4.8	4.3	5.0

[a] Range of 0–10.
[b] Oxidation time at 60°C. in presence of oxygen.

TABLE III

Dimer Content of Vegetable Oils

Oil	Flavor Score		Dimer
	Initial	4-Day storage	
			%
Soybean			
A	8.1	6.2	1.0
B	6.5	5.7	1.4
C	8.1	4.6	1.7
E	7.0	5.4	1.6
F	8.0	6.4	1.7
G	7.2	5.1	2.0
Safflower			
A	8.3	7.2	1.0
B	7.5	6.1	1.4
C	8.8	5.9	2.9
D	8.5	5.5	3.7
Cottonseed			
A	6.9	5.5	1.5
B	8.2	5.7	2.3
C	8.5	5.5	2.6
Corn	.....	.....	1.5

Although these oils exhibit a wide difference in oxidative and flavor stabilities, there is no great variation in their dimer contents. Variation in the chemical nature of dimers from various fatty acid hydroperoxides may cause a corresponding variation in their relative stability to oxidation and decomposition. Further studies on the influence of fat dimers on oil stability are in progress at this laboratory.

Since thermal polymerization possibly occurs during high-temperature deodorization of edible oils, the chromatographic method of analysis was applied to this problem. Conjugated and nonconjugated fatty acids and their methyl esters (free of peroxides) were polymerized by heating at 290°C. in sealed evacuated tubes. Chromatographic analysis of thermal polymers was achieved under the same conditions as those used for oxidative polymers. Figure 5 shows that the thermal polymeric acids are eluted in the same position as the oxidative-thermal dimers. Thus, when free acids are chromatographed, the liquid-partition chromatographic method does not distinguish between oxidative-thermal (hydroperoxide) and true thermal dimers. Lack of model compounds, representing oxidative dimers and true thermal dimers, has handicapped our attempts to separate these two chromatographically.

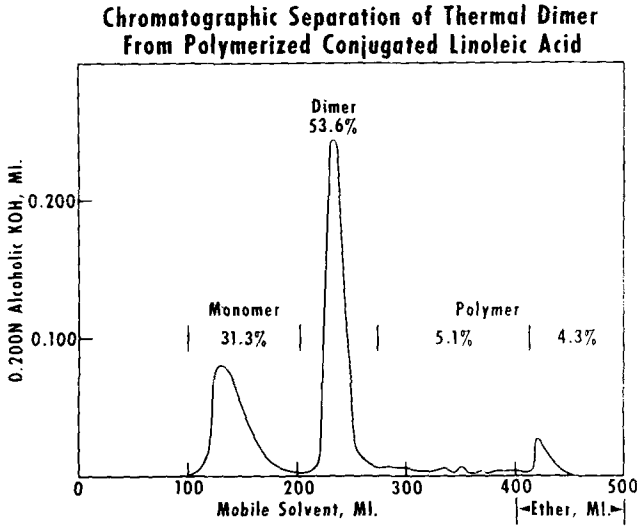

FIG. 5. Chromatographic separation of thermal dimer and polymer from conjugated linoleic acid polymerized at 290°C. for 4 hrs.

Table IV shows the extent of polymerization occurring in fatty acids and their methyl esters when heated at 290°C. in sealed evacuated tubes. The greater polymerization occurring in alkali-conjugated acids and esters than in normal nonconjugated acids agrees with the findings of Paschke and Wheeler (9). A kinetic study indicates that the loss of monomeric material in methyl linoleate follows initially a first-order reaction ($k_1 = 0.039$ hr.$^{-1}$). In the alkali-conjugated methyl linoleate, monomer loss follows second-order kinetics ($k^2 = 0.00269\%^{-1}$ hr.$^{-1}$). These results conform to the mechanism advanced by Paschke and Wheeler that in normal nonconjugated fatty esters the rate-determining step is the first-order, unimolecular conjugation of the esters whereas in alkali-conjugated methyl esters the rate-determining step is the second-order, bimolecular dimerization reaction.

Many applications exist for the chromatographic technique of dimer analysis both in the edible oil field and in industrial uses of glyceride oils. The method has a greater sensitivity than distillation and determines small amounts of either monomer or dimer in the presence of larger amounts of one over the other.

TABLE IV
Thermal Polymerization of Fatty Acids and Their Methyl Esters

Heating time at 290°C.	Monomer	Polymer	Polar material
hr.	%	%	%
Methyl linoleate			
3	87.8	10.0	0.6
6	82.6	15.9	0.6
12	61.8	30.7	4.0
24	35.9	54.9	6.8
Alkali-conjugated methyl linoleate [a]			
2	67.9	22.2	4.4
4.5	47.4	42.7	6.9
6	41.8	49.9	8.2
8	34.7	54.4	8.2
Linoleic acid			
4	64.8	29.3	4.2
8	42.6	47.9	6.3
12	36.2	54.3	8.3
Alkali-conjugated linoleic acid [a]			
0	97.2	3.2	.....
1	58.1	34.1	5.3
2	40.1	45.2	9.4
4	31.3	53.6	9.4
6	27.4	53.0	15.4

[a] k 232 mμ = 77.0 (on methyl ester basis).

The method may be used to study basic fat processing and to follow compositional changes that occur in oils subjected to heat and deep fat-frying conditions. Other fields of applying the technique lie in studies on films and film-forming properties of oils produced by autoxidation and polymerization of heated or blown oils.

Acknowledgment

C.R. Scholfield supplied the alkali-conjugated linoleic acids.

REFERENCES

1. Braae, E., Chem. and Ind. (London), 1152 (1958).
2. Evans, C.D., Frankel, E.N., Cooney, Patricia M., and Moser, Helen A., J. Am. Oil Chemists' Soc., *37*, 452 (1960).
3. Frankel, E.N., Evans, C.D., and Cowan, J.C., J. Am. Oil Chemists' Soc., *37*, 418 (1960).
4. Frankel, E.N., Evans, C.D., McConnell, D.G., and Jones, E.P., J. Am. Oil Chemists' Soc., *38*, 134–137 (1961).
5. Henick, A.S., Benca, M.F., and Mitchell, H.H., J. Am. Oil Chemists' Soc., *31*, 88–91 (1954).
6. Holm, U., Ekbom, K., and Wode, G., J. Am. Oil Chemists' Soc., *34*, 606–609 (1957).
7. Jones, E.P., and Stolp, J.A., J. Am. Oil Chemists' Soc., *35*, 71–76 (1958).
8. Moser, Helen A., Dutton, H.J., Evans, C.D., and Cowan, J.C., Food Technol., *4*, 105 (1950).
9. Paschke, R.F., Jackson, J.E., and Wheeler, D.H., Ind. Eng. Chem., *44*, 1113–1118 (1952).
10. van Duin, H., Nature, *180*, 1473 (1957).

[Received July 27, 1960]

Analyses of Lipids and Oxidation Products by Partition Chromatography. Fatty Acid Hydroperoxides

E.N. FRANKEL, C.D. EVANS, D.G. McCONNELL, and E.P. JONES,
Northern Regional Research Laboratory,[1] Peoria, Illinois

A liquid partition chromatographic method was developed to isolate and determine hydroperoxides in autoxidized fatty acids or their methyl esters. By the use of benzene containing 2 to 4% methanol as the mobile solvent, the hydroperoxides were separated from unoxidized fatty acids or methyl esters and from secondary and polymeric decomposition products. In the analyses of oxidized fatty acids, diethyl ether was necessary to elute the secondary decomposition products.

Saponification of autoxidized fatty esters destroyed the peroxides as determined iodometrically, but the resulting acids contained a fraction which was eluted in the same position as hydroperoxide acids. Evidence showed that this fraction is a monomeric hydroxy fatty acid containing conjugated *cis-trans* and *trans-trans* unsaturation.

Fatty ester hydroperoxides were isolated chromatographically in yields and purity comparable to those reported in the literature by countercurrent distribution. The concentrations of methyl linoleate hydroperoxide determined chromatographically were smaller than indicated by the peroxide value and diene conjugation of the autoxidized methyl linoleate.

ISOLATION of pure fat hydroperoxides is one of the most difficult steps in the elucidation of the mechanism of fat autoxidation. Early workers in this field obtained hydroperoxide concentrates by various methods, including molecular distillation (9) low-temperature crystallization (20), and adsorption chromatography (2,3,8,9). Those procedures generally give low yields of hydroperoxides because of varying degrees of decomposition. More recently, purer hydroperoxide concentrates were obtained in higher yields by countercurrent solvent distribution (5,13,14,17,22), urea fractionation (7), and reverse-phase partition chromatography (4,18).

This second paper in the series presents a liquid partition chromatographic method for the isolation and determination of pure fatty esters or fatty acid hydroperoxides. This method has proved useful for routine analyses and for isolations in fat autoxidation studies. The method was also applied to the determination of dimeric and polymeric products in oils (12), hydroxy fatty acids and esters, and partial glycerides (10).

Experimental

The fatty acids and their methyl esters used in this study were obtained from the Hormel Institute. Saponification of oxidized methyl esters was carried out according to the A.O.C.S. Method Ca 6b-53, and the acids were obtained by ether extraction of the acidified soaps.

The procedures for chromatography and titration of acids were the same as described for the determination of dimeric and polymeric acids (12). In highly oxidized fatty acid samples, ethyl ether was added to the column at the end of the run in order to elute the polar fraction of secondary oxidation products. The chromatographic fractionation of methyl esters was followed by collecting fractions in tared 10-ml. beakers, evaporating the solvent on a steam plate, and drying their contents to constant weight in a desiccator.

Autoxidations were carried out at 37°C. on 1- to

[1] This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture.