frying oils during normal processing. (ii) what particular fractions are toxic and (iii) what are the effects of long term feeding of oxidised fats.

The papers presented at this symposium are not intended to review all the work but to present further observations to elucidate the questions.

REFERENCES

1. Perkins, E. G., Food Tech. 508 (1960).
2. Symposium on Foods: Lipids and Their Oxidation, Editor, H. W. Schultz, Avi Publishing Co. Inc., Westport, Conn. (1962).
3. Kaunitz, H., Nature (London) *197*, 600 (1963).
4. Kritchevesky, D., S. A. Tepper and J. Langan, J. Nutr. *77*, 127 (1962).
5. Wills, E. D., Biochem. Pharmacol. *7*, 7 (1961).
6. Desai, I. D., and A. L. Tappel, J. Lipid Res. *4*, 204 (1963).
7. Anon, A., Nutr. Rev. *20*, 346 (1963).
8. Sugai, M., L. A. Witting, H. Tsuchiyama and F. A. Kummerow, Cancer Res. *22*, 510 (1962).
9. Warner, W. D., P. N. Abell, P. E. Mone, C. E. Polling and E. E. Rice, J. Am. Diet Assoc. *40*, 422 (1962).
10. Lea, C. H., L. J. Parr, J. L. Estrange and K. J. Carpenter, Brit. J. Nutr. *18*, 369 (1964).
11. Custot, F., Ann. Nutr. Aliment. *13*, A417 (1959).
12. Fleishman, A. I., A. Florin, J. Fitzgerald, A. B. Caldwell and G. Eastwood, J. Am. Diet. Assoc. *42*, 394 (1963).
13. Sahasrabudhe, M. R., and I. G. Farn, JAOCS *41*, 264 (1964).

Chromatographic Studies on Oxidative and Thermal Fatty Acid Dimers[1]

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

Abstract

A chromatographic study was carried out to investigate the nature of polymeric products in edible oils. Dimers from low-temp oxidation of methyl linoleate were compared with thermal dimers from high-temp polymerization of conjugated methyl linoleate. The distilled dimers were subjected to liquid-partition chromatographic separations on silicic acid columns as methyl esters, as free acids, and as methyl esters prepared by saponification and reesterification. Chromatographically isolated dimer fractions were also rechromatographed before and after each treatment.

When thermal dimer esters are saponified and reesterified, chromatographic recoveries are quantitative, and the expected changes in polarity result; whereas, with oxidative dimer esters, gross changes in polarity occur. Chromatographic separations of dimer esters or their acids fractionate into distinct areas of increasing polarity.

Introduction

POLYMERIC MATERIALS in glyceride oils may result from thermal treatment, oxidation, or a combination of both. Some of the most effective catalysts that cause dimerization of fatty acids are those that generate free radicals. UV light, peroxides, anthraquinones and metals in the presence or absence of air induce polymerization. High temps and the absence of air are required to form thermal polymers. Although the composition and structure of the thermal and dehydro fatty acid polymers have been well characterized, the structure of polymers formed during active oxidation is unknown and their composition varies. Studies designed to characterize oxidative polymers have relied upon distillation, solvent fractionation, selective adsorption or chromatographic separations to isolate a homogeneous material suitable for analysis.

Polymer formation in edible fats concerns the oil processor, the food technologist, the nutritionist and the consumer because of the changes polymers induce in the properties and characteristics of the fat. In 1960 Perkins (19) reviewed the literature on the chemical and nutritional changes that occur in heated fats. Firestone (9) in 1963 reviewed the methods for the determination of polymers in fats and oils. A method for the determination of 0.01 to 1.0% thermal dimer was published by Rost (21,22); but he states that it is not suitable for the determination of oxidatively derived polymers in fats, and cautions that oxidation of the thermal polymer through exposure to air must be avoided. We have described a method of partition chromatography using silicic acid to separate either thermal or oxidative polymeric fatty acids from the unaltered natural acids (12). Structural differences between the thermal and oxidative type of polymers and the presence of polar groups in the oxidative-type polymer indicate that a difference in chromatographic polarity should exist. The ability to distinguish between thermal and oxidative polymers would contribute to a better understanding of the behavior of fats and oils in industrial and edible applications.

The present paper describes chromatographic studies designed to distinguish between thermal and oxidative dimers by the analysis of their methyl esters, the free dimeric acids obtained by saponification of the esters, and the esters obtained by reesterification of isolated dimeric acids.

Experimental

Materials. Methyl linoleate used for the preparation of the oxidative dimer was obtained by esterification of a linoleic acid conc obtained from safflower fatty acids through Podbielniak extraction with furfural and hexane (3). The fraction boiling at 147–150C at 0.5 mm was used, which by gas-liquid chromatography (GLC) showed a purity of 98.8% and the presence of 1.2% methyl oleate. The alkali-conjugated methyl linoleate was prepared from safflower fatty acids obtained through crystallization in hexane at −40C. Isomerization was conducted for 45 min at 190–200C in an ethylene glycol solution containing 15% potassium hydroxide. The acids were methylated in methanol and H_2SO_4 and purified by distillation at 144–150C 0.4 mm. The conjugated methyl linoleate esters have an absorptivity of 76.9 at 232 mμ, indicating a conjugation of 82%. Soybean methyl esters were prepared from refined soybean oil by transesterification with sodium methoxide. At the start of each experiment esters were freshly distilled.

Autoxidation. Methyl linoleate was oxidized without catalyst at 25C in a closed oxygen system at atmo-

[1] Presented at the AOCS Meeting, New Orleans, 1964. Honorable Mention, Bond Award Competition.
[2] A laboratory of the No. Utiliz. Res. & Dev. Div., ARS, USDA.

TABLE I

Distillation of Polymers

Fraction	Temp °C	Oxidative		Thermal	
		Yield %	Isolated *trans* %	Yield %	Isolated *trans* %
Monomer	140	17	15.5	39	14.9
Dimer	200	50	19.1	47	22.3
Trimer	250	20	18.2	...	...
Residue	<250	13	16.6	14	20.3

spheric pressure to peroxide levels of approx 500 meq/kg. The methyl ester hydroperoxides were concd by the countercurrent extraction procedure of Zilch et al. (27) employing the solvent system of 80% aqueous ethanol and petroleum ether. The recovered unoxidized esters were again subjected to oxidation and the hydroperoxides extracted. The combined methyl linoleate hydroperoxides had a peroxide level of 5,200 (theoretical value for the pure monohydroperoxide of methyl linoleate is 6126 meq).

Soybean methyl esters were oxidized at 6C by bubbling oxygen through a sintered-glass filter stick submerged in the sample. The esters were oxidized to a peroxide level of 1,400 in 144 hr. During the working day the esters were exposed to UV radiation from a 100-w mercury vapor lamp. Hydroperoxides were not extracted from the unoxidized soybean methyl esters, and the entire mixture was used for polymerization.

Polymerization. The purified methyl linoleate hydroperoxide esters were polymerized in 20-g batches in evacuated and sealed flasks made from 100 ml distilling bulbs. Flasks were completely immersed in a 200C oil bath and held for 20 min. A similar technique was used for the thermal polymerization of conjugated methyl linoleate except the time was extended to seven hr at 290C. Before sealing, the polymerization flasks were subjected to repeated thawing and freezing (under dry ice) to insure complete removal of any dissolved air or oxygen. The autoxidized soybean methyl esters were polymerized under nitrogen at atmospheric pressure for 30 min at 210C. Nitrogen was continuously passed through the esters during the heating and cooling cycle to blanket the samples from air and serve as a means of collecting volatiles for autoxidation studies.

Distillation. Esters from several polymerization flasks were combined and after thorough degassing were subjected to molecular distillation in the Asco "50" Roto film still. Monomers were distilled at 5–10 μ by the first pass at 140C, the dimers and trimers by the second and third passes at 200 and 250C, respectively.

Saponification and Reesterification. Polymers and subfractions were saponified according to AOCS method Cd-3-25 (1). Reesterification was carried out with dimethoxy propane (DMP) at room temp according to the method of Radin and Hajra (20). A few subfractions were also reesterified with diazomethane, and the results agreed with those obtained by the DMP procedure.

Chromatography. The liquid-partition benzene-methanol system previously described (11,12) was used for the various fractionations. All silicic acid chromatographic columns were prepared with an immobile solvent of 16% by wt methanol in benzene and an eluting solvent of 2% methanolic benzene. To remove the highly polar materials when esters are chromatographed, diethyl ether was added after 350 ml of mobile solvent had passed through the column. Recovery of the sample from the column was almost quantitative, except for the monomeric fraction obtained from the oxidative polymerizations.

TABLE II

Hydroxyl Content of Fractions from Methyl Linoleate Oxidative Dimer

Chromatographic fraction	Hydroxyl %	
	Run A	Run B
Peak I	Trace	Trace
Peak II	27.1	18.5
Peak III	48.7	57.4
Peak IV (ether)	123.5[a]	123.0[a]
Original dimer	29.9	32.1

[a] Methyl ricinoleate = 100%.

Mol wt of the polymers were determined with a Mechrolab vapor pressure osmometer, Model 301. Temp depression readings were taken for several different polymer concn in benzene, and by extrapolating to a zero concn the number-average mol wt was obtained. Isolated *trans* values were determined in carbon disulfide solutions by IR absorption at 10.3 μ and expressed as elaidate. Hydroxyl contents were determined by absorption at 2.86 μ and reported as a percentage of the absorption shown by pure methyl ricinoleate (13). Hydroperoxide groups are completely destroyed during dimer preparation and therefore offer no interference in the spectral method.

Results

Oxidative and thermal dimers prepared from methyl linoleate were typical polymeric products described previously (10,12); and their preparation, distillation and characterization offered no particular problems. Table I shows the distillation yields of the various fractions and their isolated *trans* contents. The mol wt and hydroxyl contents of the respective fractions are reported in Tables II and III.

Oxidative Dimers. The hydroxyl content of the oxidative dimer shows a close relationship to the polarity of the various chromatographic fractions. The similarity in analysis of two oxidative dimers, prepared several months apart, is shown by runs A and B in Table II. Although not an exact duplication, the results do indicate that a fairly reproducible dimeric material can be prepared by rapid thermal decomposition of fatty acid hydroperoxides. Hydroxyl analyses are based on methyl ricinoleate and results of 123% indicate 1.23 times as many hydroxyls as the standard. The mol wt of the distilled polymers are in the expected ranges and show good agreement with the values previously reported in the literature (10,21). The mol wt of the four oxidative dimeric fractions obtained by liquid-partition chromatography are slightly lower than theoretical values, but clearly indicate the dimeric nature of all fractions. Mol wt of the saponified and reesterified dimer fractions are almost the expected theoretical values. Slight increases in mol wt would be expected in any lactones, epoxides or other cyclic groups, and any free acids were present in the polymers and therefore available for esterification. Reasons for the apparently large increase in

TABLE III

Mol Wt of Polymers

Type	Distilled ester	Chromatographed ester	Distilled ester saponified and reesterified
Thermal			
Monomer	265	.....	.....
Dimer	590	.....	.....
Residue	840	.....	.....
Oxidative			
Monomer	275	I[a] 546	465
Dimer	560 ⟷	II 570	600
Trimer	840	III 586	1085
Residue	1930	IV 540	1660

[a] Peak I, of chromatographed dimer.

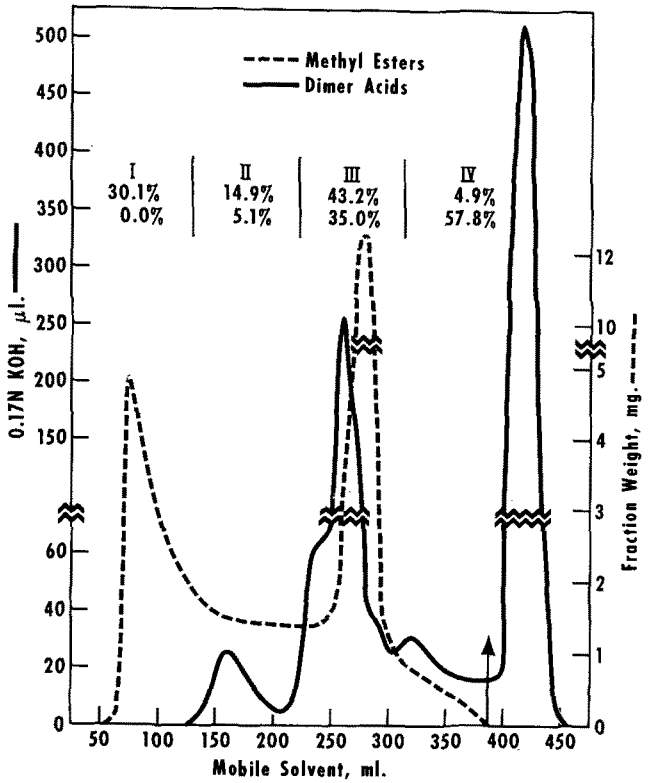

FIG. 1. Chromatograms of oxidative methyl linoleate dimers as esters and after saponification as free acids. Arrow indicates when diethyl ether added as eluting solvent.

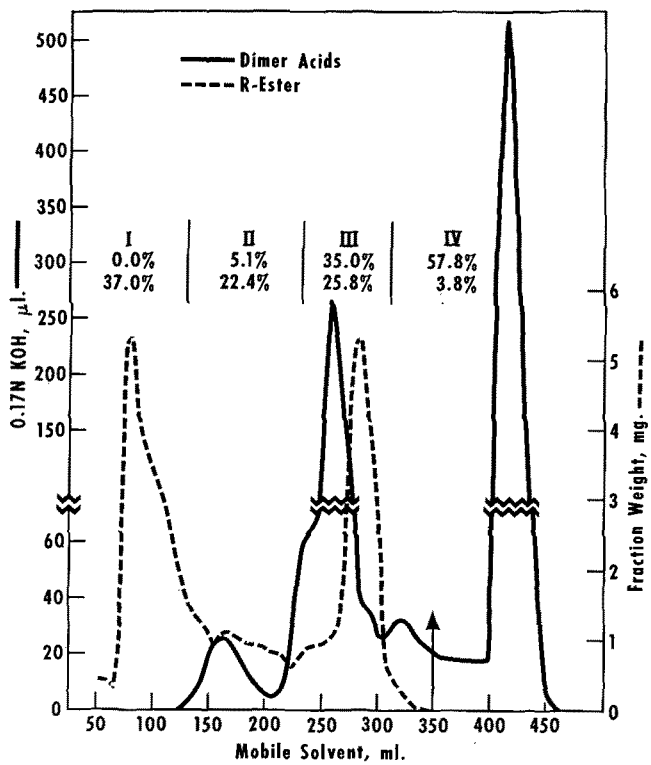

FIG. 2. Chromatograms of oxidative methyl linoleate dimers as free acids and after reesterification (R-ester).

mol wt of the trimer fraction are not known. Probably the increase in mol wt of monomeric fraction results from a loss of the short-chain components. Reduction in mol wt of residue material also probably results through the splitting of intramolecular esters of polymeric units.

Figures 1 and 2 show the separation obtained for the oxidative dimer when prepared, distilled and chromatographed 1) as the methyl esters, 2) after saponification of the methyl esters, and 3) after reesterification of the isolated acids. Four distinct chromatographic areas can be seen at different elution volumes: Peak I below 125 ml; peak II 125–220 ml; peak III 220–350 ml; and peak IV in the neighborhood of 400 ml. Oxidative esters show well-resolved peaks in areas I and III, and an appreciable amt of unresolved material in the peak II area. Dimeric acids show better resolution of fractions in peak areas II, III, and IV. The quantities of an oxidative dimer in each of the acid-chromatogram peaks bear no simple relationship to its distribution in the ester chromatogram. Reesterification of either the entire saponified oxidative dimer-acid mixture or the individual fractionated acids does not result in the identical products(s) from which the polymeric acids are obtained (Figs. 1 and 2, and Table IV). Reesterification of the oxidative dimer acids gives an approx chromatographic redistribution to the original ester. However, when isolated chromatographic fractions or subfractions of oxidative dimers are similarly treated by saponification and reesterification, the initial distribution of the fractions is not attained as shown in Table IV.

The monomeric fraction from distillation of the oxidative polymer is exceedingly complex and contains a wide distribution of polar materials. Repeated attempts to raise the column recovery failed; even washing with methanol followed by aqueous hydrochloric acid did not give complete recovery. Some loss, ca. 10–15%, is known to occur on drying the eluted materials to constant wt. GLC analysis of the monomer shows some 15 peaks eluted by temp programming to 240C. Characterizations of these components have not been undertaken.

Extended studies have not been made on the trimer and residue fractions. As shown in Table IV, trimeric material behaves chromatographically much like dimeric material. Increased amt of the highly polar fractions are found in the trimer and residue fractions of both oxidative and thermal dimers. Shorter chain secondary oxidation products and scission acids incorporated into the polymeric material could account for this increased chromatographic polarity.

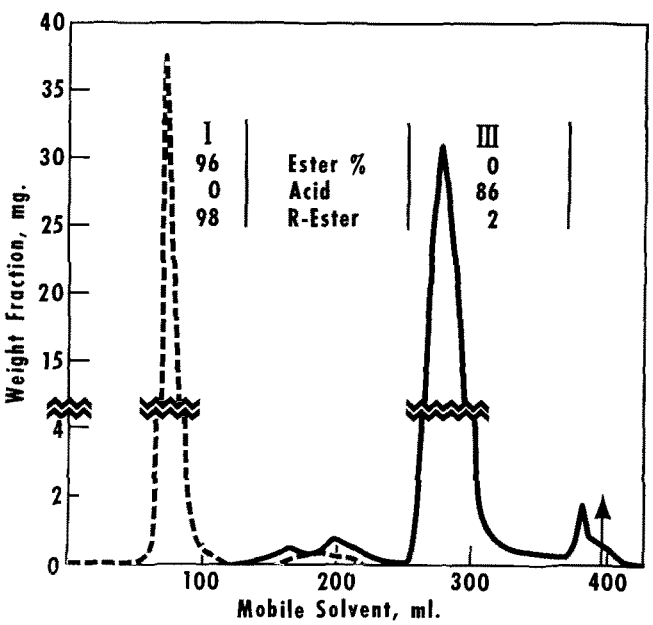

FIG. 3. Chromatograms of thermal methyl linoleate dimers as esters, as free acids after saponification, and as esters after reesterification (R-ester). The two ester curves coincide.

TABLE IV
Chromatographic Fractionation of Oxidative and Thermal Polymers

Chromatogram	Peak area I %	II %	III %	IV %	Column recovery %
	Oxidative polymers				
Monomer					
Ester	20	25	14	3	62
Acid	.....	12	44	51	107
Reesterified	20	31	33	4	88
Dimer					
Acid	34	26	36	5	101
Ester	.....	4	42	50	96
Reesterified	31	29	34	5	99
Trimer					
Ester	24	27	44	6	101
Acid	.....	2	26	72	100
Reesterified	15	22	46	6	89
Residue					
Ester	(←26.1→)		59.1	12.2	97.9
Acid	Mostly insoluble in mobile solvent				
Reesterified	(←22.6→)		56.1	10.4	89.1
	Thermal polymers				
Monomer					
Ester	100	2.1	0.9	2.1	105.1
Acid	.....	92.9	4.2	1.7	99.3
Reesterified	73.6	4.3	5.5	2.5	85.8
Dimer					
Ester	95.8	1.8	.....	1.4	99
Acid	.....	4.7	85.5	5.6	96
Reesterified	98.2	.....	1.7	1.0	100.9
Residue					
Ester	94.4	2.1	1.7	1.3	99.5
Acid	.....	1.4	62.3	28.4	92.1
Reesterified	84.0	(←16.4→)		1.5	101.9
	Subfractions of oxidative dimer				
Dimer ester	29.3	13.8	39.3	4.3	86.6
Peak I	[29.3]				
Saponified	.....	8.0	47.4	40.0	95.4
Reesterified	67.2	15.6	17.1	1.6	97.0
Peak III			[39.3]		
Saponified	.....	.....	17.9	7.6 + 70.9	96.4
Reesterified	19.0	15.1	49.5	13.5	97.1
Subpeak III			[17.9]		
Reesterified	30.3	35.7	33.7	2.9	102.6
Subpeak IVa				[7.6]	
Reesterified	28.3	44.9	26.7	6.5	107.3
Subpeak IVb				[70.9]	
Reesterified	3.1	15.5	67.2	10.8	96.5
	Subfractions of thermal dimer				
Dimer ester	95.8	1.8	.....	1.4	99.0
Peak I	[95.8]				
Saponified	.....	1.5	79.1	14.6	95.2
Reesterified	99.3	.....	.....	1.7	101.0
Dimer acid	.....	4.7	85.5	5.6	95.8
Peak III			[85.5]		
Reesterified	95.3	.....	.....	2.1	98.6
	1:1 Mixture of oxidative and thermal polymers				
Dimer 1:1					
Esters	58.6	12.8	20.3	2.8	94.5
Acids	.....	4.8	58.4	31.5	94.8
Reesterified	61.6	9.9	21.0	4.2	96.3
	Theoretical for 1:1 mixture				
Dimer 1:1					
Esters	62.6	7.8	19.7	2.8	.....
Acids	.....	5.0	60.2	31.7	.....
Reesterified	67.6	11.2	13.8	2.4	.....

Thermal Dimers. Dimers prepared by thermal polymerization of the conjugated esters and subjected to saponification and reesterification behave chromatographically in the expected fashion according to their polarities. Figure 3 shows that the nonpolar esters are exclusively in peak I with less than 2% in peak II.

These minor components in peak II are either monomeric free acids or half esters because they disappear on reesterification. Dimer esters can be freed of small amt of acid constituents by passage through ion-exchange resins. Upon saponification, the dimer acids move quantitatively to peak III with a small percentage which probably results from oxidation in the most polar area. Upon reesterification of the acids, the dimeric material again moves quantitatively (98.2%) back to peak I. Only one ester curve is shown in Figure 3 since the ester and reesterified curves coincide.

The monomeric fraction obtained through distillation of the thermal polymerization products contains unpolymerized acids and scission acids with less than 4.2% of any polymeric acid. Data presented in Table IV show 92.9% are nonpolymeric acids (peak II), and although the total recovery upon reesterification is not quantitative, there is no doubt about the chemical nature of these acids. GLC analysis of the reesterified monomeric fraction shows eight peaks of which linoleic acid and two unknown components constitute over 80% of the material.

Chromatographic analysis of the residue fraction indicates a high percentage of polymeric acids—62.3% in peak III, plus a large fraction (28.4%) of acids more polar than polymeric acids. This latter fraction probably results mostly from fission or cracking of the fatty acid chain, since all polymeric acids having a ratio of 1 carboxyl group/18 carbons always chromatograph in peak III. Trace oxidation occurring during preparation of esters or polymers would also contribute to this fraction.

Chromatographic Subfractionation. Subfraction data on oxidative dimers (Table IV) indicate that chromatographically separated fractions are not homogeneous and, upon saponification and reesterification, split into fractions of various polarities. The nonpolar peak I components of an oxidative dimer would be expected to behave chromatographically, and perhaps chemically, like a thermal or dehydro dimer. However, upon saponification of peak I (29.3% of the dimer) the polymeric acids were divided almost equally between peak areas III (47.4%) and IV (40.0%). Reesterification of the oxidative dimeric acids returns only 67.2% to the nonpolar peak I area, whereas for true thermal dimers reesterification returns 98+% to peak I.

When peak III, the major oxidative dimer ester component, is subfractionated after saponification (Fig. 4) two distinct acid components (7.6% + 70.9%)

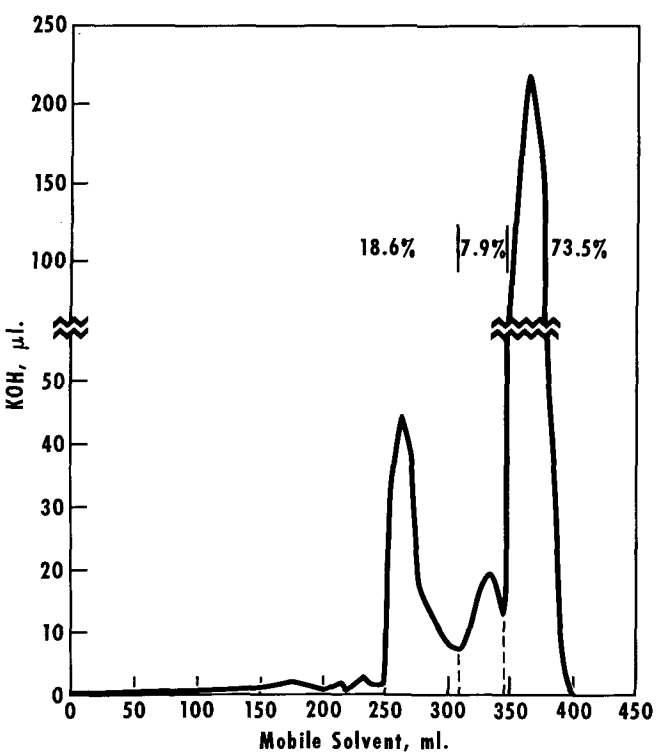

FIG. 4. Chromatogram of isolated peak III of oxidative methyl linoleate dimer after saponification and chromatographed as dimer acids.

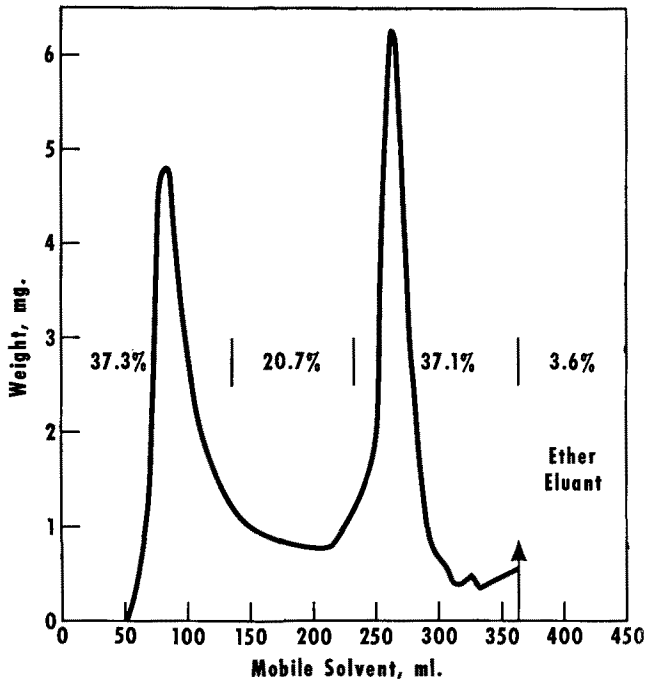

FIG. 5. Chromatogram of oxidative soybean oil methyl ester dimers.

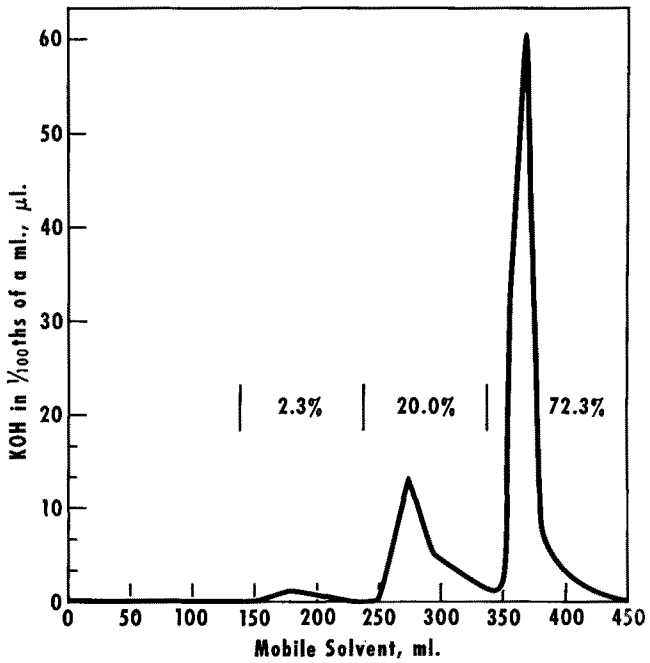

FIG. 6. Chromatogram of oxidative soybean oil methyl ester dimers after saponification.

occur in the peak IV area and a small amt (17.9%) remains in the peak III area. Thus 78.5% of the ester is composed of highly polar fatty acids. When these acids (all from peak III ester) are reesterified, a redistribution occurs to all four peak areas; i.e., 19.0, 15.1, 49.5 and 13.5%, respectively. In this subfraction of the oxidative dimer, only 49.5% returned to the original chromatographic area and polarity of the starting ester. The absence of any peak III in thermal esters does not allow a comparison, but because of the total absence of any thermal ester in this area, it is possible to distinguish between thermal and oxidative dimers by the ester concn found at peak III.

When the polar acids, obtained by saponification of peak III oxidative dimer esters, are reesterified and chromatographed, fractions appear in each of the four peak areas. A greater concn of components in the nonpolar peaks results from saponification and reesterification of individual fractions of the lowest polarity. Thus the three acid subfractions (17.9, 7.6 and 70.9%) show components having peak I polarities of 30.3, 28.3 and 3.1%, respectively. Although the most polar acid subfraction (70.9%) originally came entirely from the peak III oxidative ester, upon reesterification it returned only 67.2% to the area of its original polarity. When the thermal dimeric acids in peaks III and IV are reesterified, they returned 95–99% to nonpolar peak I.

Separation of Mixed Oxidative and Thermal Dimers. Equal wt mixtures of the methyl linoleate thermal dimer and of the methyl linoleate oxidative dimer were chromatographed as esters and as free acids after saponification and after reesterification. These chromatographic results shown at the end of Table IV, agree closely to the expected fractionation as calculated from the chromatographic separations of the original dimers. As indicated previously, the true thermal dimer shows no ester components in peak III and the oxidative dimer will show approx 40% wt distribution in the peak III area. Since the 20.3% peak III ester fraction equals approx 40% of the oxidative dimer, the mixture contains 50% of that dimer. Agreement in known systems with the amt of added thermal dimer indicates the possibility of developing a chromatographic method suitable for determining the respective amt of oxidative and thermal dimers in the presence of each other. It is believed that such a chromatographic method is feasible based on the polarity differences between the two.

Figures 5–7 show the behavior of oxidative dimers made from soybean methyl esters. Since linoleic makes up 52% of soybean oil, fair agreement would be expected with dimers made from pure linoleate esters. Good agreement exists between soybean ester dimers and methyl linoleate dimers on the chromatographic distribution of the components within the four polar fractions. Soybean dimeric acids show a considerably higher proportion (72.3%) of material in the highly polar peak IV area. This percentage might be expected because both the high levels of oxidation and the oxidation of linolenic acid lead to higher values in the extremely polar fraction (11). Changes of this type in the chromatographic distribution of polymer components indicate the need of detailed knowledge on the effect of conditions of oxidation and hydro-

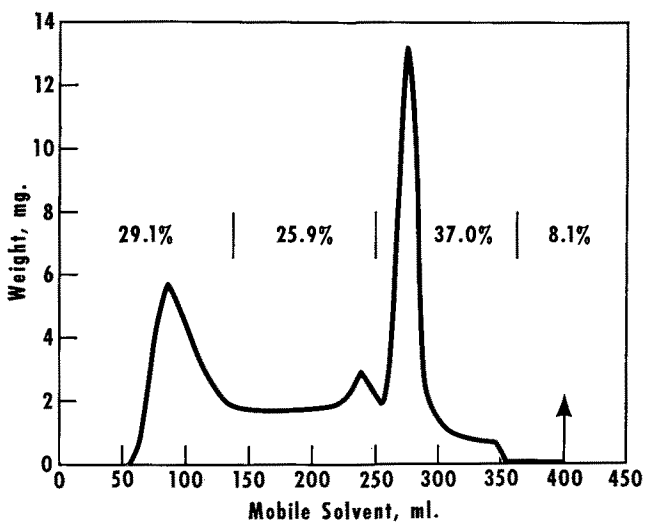

FIG. 7. Chromatogram of oxidative soybean methyl ester dimer after saponification and reesterification.

peroxide decompositions, as well as fat composition on the polarity of the oxidation products.

Discussion

Application of a chromatographic method to the determination of oxidative and thermal dimers has been discussed in previous publications (7,12). When acid dimers are chromatographed, the effects of the less polar carbonyl and hydroxyl groups are largely depressed. When chromatographed as acids, both oxidatively and thermally prepared fatty acid dimers are eluted from a chromatographic column in the same position. However, if these dimers are chromatographed as esters, the strong polar effect of the acid groups is depressed, and the polarity of the carbonyl and hydroxyl groups determines the position or elution-volume of the esters. The elution-volume of thermal dimers esters having no polar groups is the same as a normal or unoxidized fatty acid ester. Oxidatively prepared dimer esters have a greater polarity because of the presence of hydroxyl, carbonyl and other oxygen-containing groups. These polarity differences are the basis for determining oxidative dimers in the presence of thermal dimers. Published chromatograms (12) of thermal or oxidative dimers show four definite peak areas where fractions are eluted. The first elution area, or the nonpolar peak (I), contains the normal esters, thermal polymer esters, hydrocarbons and similar nonpolar materials. The second elution area (II), which is less defined, contains mildly polar materials, probably keto esters, epoxy esters, hydroxy fatty acid esters and similar products. In the peak III area, the concn of methanol in the eluant increases sharply, and the polar materials are coned into a narrow band and quickly eluated. This peak includes the dimer acids, hydroxy acids and hydroperoxidic acids, if any are present. The relationship of polarity to elution-volume of the various acid and ester dimeric materials is depicted in Table V.

Considerable confusion exists in the literature on the terminology and description of fat polymeric materials. Fixed definitions are not yet possible, but thermal dimers imply that polymerization has occurred in the absence of air. These thermal polymers will contain various cyclic Diels-Alder addition products, noncyclic dehydro polymers of various structures, and perhaps hybrid dimers in which one of the monomeric units has cyclized before dimerization occurs. Oxidative polymer implies that polymerization takes place in the presence of active oxygen, where probably the first reaction is the formation of monomeric hydroperoxides. When heat is applied during oxidation, polymerization reactions become exceedingly complex, not only through free radical reactions of the decomposing hydroperoxides, but by simultaneous formation of thermal dimers and through combinations of different active monomeric materials, many of which will contain oxygen. Polarity of the dimeric material, which largely depends on oxygen-containing groups, may in part be affected by the temp of polymerization and of hydroperoxide decomposition. Fedeli et al. (8) report that thermal polymerization of vegetable oils at temp up to 260C involves linoleic acid solely and that linolenic acid becomes involved only at temp above 280C. In studies on thermal scission of cod-liver oil peroxides, Aure et al. (2) report that at temp below 125C conjugation and polymerization are avoided. They also reported that unsaturated aldehydes begin to polymerize at 150C and that the rate increases rapidly at higher temp. Johnson et al. (16) found that methyl linoleate hydroperoxides decompose and form polymers, even when stored under nitrogen at 0C. Williamson (26) decomposed methyl linoleate hydroperoxide by continous heating at 100C for 23 hr. The polymer obtained after molecular distillation was free of acid groups but contained hydroxyl, carbonyl and epoxide groups; and he noted that chromatographic separation was impractical.

Through solvent fraction and distillation, the three types of polymer fractions obtained by Williamson were: 1) dimers carbon-to-carbon linked that contained no hydroxyl groups and retained a large proportion of the original fatty acid unsaturation; 2) dimers carbon-to-carbon linked that contained an appreciably hydroxyl content and had a relatively low degree of unsaturation; and 3) trimers containing a higher proportion of oxygen than the dimers and a high degree of unsaturation. Chang and Kummerow (5) oxidized ethyl linoleate at 30C and obtained a series of polymeric fractions of increasing mol wt by solvent fractionation. The unheated polymeric fractions were depolymerized by strong acids, and characterization of the split products indicated that the oxidative polymers were joined by carbon-to-oxygen bonds. Swern et al. (24) obtained 30–40% yield of polymers from 65C air oxidation of methyl oleate and concluded that the polymers obtained were oxygen-linked, probably as ethers since the polymers were not saponifiable to monomeric units.

Saponification of fats and fatty products is somewhat an arbitrary procedure, and for the more difficult saponifications, higher boiling solvents and longer times of saponification have been employed. The difficulty of saponifying paint films and drying oils is well-known (23). Steric hindrance within oxidative polymers may contribute to the difficulties of saponification. Gould (14) states that it is likely that acid-catalyzed hydrolysis, esterification and saponification are subject to virtually the same steric effects. Rates of esterification are known to be goverened primarily by the total number of substituents in the α and β positions (17). Since hydroperoxide decomposition is through a free radical mechanism, it is quite likely that some hydrogen abstraction may occur on the α and β carbons of the fatty acid chain, as well as in the allyl position of the double bonds. Dimers derived from hydroperoxides in this manner would be nonpolar and behave chromatographically like thermal dimers or the dehydro polymers.

Dehydro polymers, as discussed by Clingman and Sutton (6) and by Wheeler and coworkers (15,18), derived through free radical decompositoin of a peroxide, would be free of oxygen and similar to the oxidatively derived nonpolar dimers that occur in the peak I area. Fatty-acid hydroperoxides are effective free radical polymerization catalysts (25), and conditions for similar action are present during fat

TABLE V

Chromatographic Separations and Properties of Oxidative and Thermal Dimers

Area	Chromatographed as	
	Esters	Acids
Peak I	Unoxidized esters, thermal dimers, no hydroxyl groups	Nonacidic material
Peak II	Intermediate material, unresolved mixture with few hydroxyl groups	Unoxidized acids
Peak III	Oxidative dimers with hydroxyl groups	Oxidative and thermal dimer acids
Peak IV	Secondary oxidation products and highly po'ar products show very strong hydroxyl absorption	Secondary oxidation products and highly polar materials

autoxidation. Previous studies (10) on distilled oxidative dimers (not chromatographically fractionated) indicate diene conjugation as high as 23%, and with double bonds randomly distributed from the C-6 to C-10 carbon atom of the fatty-acid chains.

Chromatographic fractionation offers a method of characterization and analysis based on polarity of the various components in oxidative polymers. Although confirming our results with the chromatographic method, Bernard and Rost (4) question the nature of the dimeric material and maintain that in normally processed soybean oil, thermal polymers constitute less than 0.1%. Since Rost's method (21,22) determines thermal polymers only, it offers a means of checking the type of dimer found in the chromatographic peak I areas obtained from the oxidative dimers.

Distilled oxidative dimers do not give highly resolved chromatographic fractions, but show a large peak of ca. 30% of the same polarity as the thermal dimer, and have a major peak approx 50% in an area of much higher polarity. Saponification may not be complete, and reesterification of these two chromatographically isolated fractions shows that they are not composed of homogenous material because fractions of the various polarities are recovered. Internal ester linkages in oxidative dimer offer a partial explanation for polarity changes where hydrolysis would release hydroxyl groups to give a polar monomeric unit within the dimer structure. Polarity of a hydroxylated dimer would be different from the original dimer.

Many parameters which influence the conditions of oxidation and hydroperoxide decomposition must be investigated, and the various interactions evaluated before any chromatographic method of dimer analysis can be fully evaluated. So far results indicate that considerable chemical and physical information regarding the composition of oxidative dimers is available through a detailed analysis of the chromatographic fractions. Temp of oxidation and the environment of peroxide breakdown are extremely important, and these two factors probably contribute most to the diversity of results recorded in the literature. Many of the usual analytical techniques need critical evaluation in dimer analysis since basic distinction of dimer types (thermal, dehydro and oxidative) are made on unsaturation, type of unsaturation, mol wt, saponification value, functional group analysis and the presence of various cyclic and heterocyclic groups. No definition of oxidative polymers is possible until these materials are fractionated and the components chemically and physically characterized. Currently it might be advantageous to define, or at least partially describe, oxidative dimers in terms of polarity as determined by some chromatographic procedure.

ACKNOWLEDGMENT

Determination of IR spectra by Mrs. Helen M. Peters; linoleic acid from R. E. Beal; and alkali-conjugated linoleic acid from C. R. Scholfield.

REFERENCES

1. AOCS Official and Tentative Methods, 2nd ed. rev., Chicago, Ill. (1962).
2. Aure, L., K. M. Anthousen and E. Bjorsvik, Rappt. Nord. Fellharskningssymp *3*, 119–23 (1961); C.A. *59*, 15506 (1963).
3. Beal, R. E., and O. L. Brekke, JAOCS *36*, 397–400 (1959).
4. Bernard, A. H., and H. E. Rost, *Ibid. 39*, 479 (1962).
5. Chang, S. S., and F. A. Kummerow, *Ibid. 30*, 403–407 (1953).
6. Clingman, A. L., and D. A. Sutton, *Ibid. 30*, 53–56 (1953).
7. Evans, C. D., Proc. Flavor Chemistry Symposium," Campbell Soup Co., 1961, pp. 123–146.
8. Fedeli, E., A. Valentini and G. Jacini, Fette, Seifen, Anstrichmittel *65*, 402–410 (1963); C.A. *59*, 5373 (1963).
9. Firestone, David, JAOCS *40*, 247–255 (1963).
10. Frankel, E. N., C. D. Evans and J. C. Cowan, *Ibid. 37*, 418–424 (1960).
11. Frankel, E. N., C. D. Evans, D. G. McConnell, E. Selke and H. J. Dutton, J. Org. Chem. *26*, 4663–4669 (1961).
12. Frankel, E. N., C. D. Evans, Helen A. Moser, D. G. McConnell and J. C. Cowan, JAOCS *38*, 130–134 (1961).
13. Frankel, E. N., D. G. McConnell and C. D. Evans, *Ibid. 39*, 297 (1962).
14. Gould. E. S., "Mechanism and Structure in Organic Chemistry," Henry Holt & Co., New York, 1959.
15. Harrison, S. A., and D. H. Wheeler, J. Am. Chem. Soc. *76*, 2379–2382 (1954).
16. Johnston, A. E., K. T. Zilch, E. Selke and H. J. Dutton, JAOCS *38*, 367–371 (1961).
17. Newman, M. S., J. Am. Chem. Soc. *72*, 4783–4786 (1950).
18. Paschke, R. F., L. E. Peterson, S. A. Harrison and D. H. Wheeler, JAOCS *41*, 56–60 (1964).
19. Perkins, E. G., Food Technol. *14*, 508–514 (1960).
20. Radin, N. S., and A. K. Hajra, J. Lipid Res. *1*, 250–251 (1960).
21. Rost, H. E., Fette, Seifen, Anstrichmittel *64*, 427–433 (1962).
22. Rost, H. E., *Ibid. 65*, 463–466 (1963).
23. Shaw, J. N., and M. W. Formo, JAOCS *31*, 448–451 (1954).
24. Swern, D., H. B. Knight, J. T. Scanlan and W. C. Ault, J. Am. Chem. Soc. *67*, 1132–1135 (1945).
25. Swern, D., J. E. Coleman, H. B. Knight, K. T. Zilch, H. J. Dutton, J. C. Cowan and J. M. Gyenge, J. Polymer Sci. *11*(5), 487–490 (1953).
26. Williamson, L., J. Appl. Chem. *3*, 301–307 (1955).
27. Zilch, K. T., H. J. Dutton and J. C. Cowan, JAOCS *29*, 244–246 (1952).

[Received September 22, 1964—Accepted November 16, 1964]

A Long-Term Nutritional Study with Fresh and Mildly Oxidized Vegetable and Animal Fats[1]

H. KAUNITZ, R. E. JOHNSON and L. PEGUS, Department of Pathology, Columbia University, New York

Abstract

Fresh and oxidized cotton seed oil (CO) olive oil (OO), chicken fat (CF) and beef fat (BF) were fed to male weanling rats for 33 to 108 weeks. Groups fed oxidized fats except OO showed a higher death rate than those fed the corresponding fresh fats. Groups fed oxidized CO and BF had the highest death rate. Histological studies of animals dying from natural causes showed more pronounced cardiac lesions in the animals fed oxidized CO. Serum, liver and brain cholesterol levels were not influenced by oxidized fats. Fatty acid composition of depot fats and of heart and liver lipids did not show significant differences between groups fed fresh and the corresponding oxidized fats.

[1] Presented at the Spring Meeting of the AOCS 1964.

IN SPITE OF THE GREAT interest in the biological effects of natural fats which have been exposed to heat and/or air, there are comparatively few reports as to any pathological findings after long-term feeding of such fats. In acute experiments, McKay (1) induced the generalized Shwartzman reaction in pregnant rats by feeding them fractions of oxidized cod liver oil during the gestation period. These episodes of disseminated intravascular clotting, which are especially noticeable in lungs and kidneys, can be prevented by the feeding of tocopherol. Raulin (2) found that the degenerative heart lesions are more frequent in rats on a low tocopherol diet. Earlier short-term studies carried out in the authors' laboratory with highly polymerized fractions of autoxidized fats revealed no recognizable histological lesions except for some edema of the gut despite the fact that the animals had enlarged livers, kidneys, and adrenals before they died (3).