Analysis of Oleate, Linoleate and Linolenate Hydroperoxides in Oxidized Ester Mixtures[1]

SEYED H. FATEMI and **EARL G. HAMMOND**, Department of Food Technology,
Iowa State University, Ames, IA 50011

ABSTRACT

The hydroperoxides in oxidized mixtures of methyl oleate, linoleate and linolenate were analyzed by reducing the hydroperoxides to the corresponding hydroxyesters and separating the hydroxyesters from the unoxidized esters by thin layer chromatography (TLC). The hydroxyesters from linolenate were separated from the other hydroxyesters by TLC on silver ion plates. The hydroxyesters were converted to TMS-hydroxy derivatives. The TMS-hydroxyoleate and TMS-hydroxylinoleate were separated by gas chromatography (GC), and all the TMS-derivatives were quantified by GC. The relative rates of oxidation of methyl oleate, linoleate and linolenate in mixtures were ca. 1:10.3:21.6. The hydroperoxides formed in the oxidation of soybean and olive oils were similar before and after randomization and similar to corresponding methyl ester mixtures.

INTRODUCTION

Comparisons of the rates of oxidation of purified esters of oleate, linoleate and linolenate have shown that these esters oxidize at rates in the ratios of 1:12:25, respectively (1,2). Because the rate of the initiation reaction in the oxidation of these esters was unknown, it is not certain what the relative rates of oxidation of these esters in mixtures would be. Wong and Hammond (3) measured the rate of oxidation of methyl oleate and linoleate in mixtures by reducing the hydroperoxides to the corresponding alcohols and separating the methyl hydroxyoleate from the methyl hydroxylinoleate by gas chromatography (GLC) of the trimethylsilyl (TMS)-derivatives. They found that the relative rates of oxidation of methyl oleate and linoleate seemed to vary with the composition of the mixture. Frankel et al. (4-7) also analyzed hydroperoxide mixtures by combined gas chromatography-mass spectrometry of the TMS-derivatives obtained from oxidized methyl esters after reduction of the hydroperoxide and saturation of the double bonds. They report that for methyl oleate the 4 expected isomers, 8, 9, 10 and 11-hydroxyoctadecanoate, are not formed in equal amounts, nor for methyl linolenate are the 4 expected isomers, 9, 12, 13 and 16-hydroxyoctadecanoate, formed in equal amounts. But the 2 isomers expected from linoleate, 9- and 13-hydroxyoctadecanoate, were produced in equal amouts. Further, they report that during the oxidation of soybean oil methyl esters, unexpectedly large amounts of the 12-hydroxyoctadecanoate are produced during the early stages of oxidation. Their data show that in the oxidation of mixtures of methyl oleate and methyl linoleate, the relative amounts of oleate and linoleate hydroperoxides are not proportional to the oleate and linoleate in the mixture. This suggests that the relative rates of oxidation of oleate and linoleate vary with the composition of the mixture.

METHODS

Methyl oleate, linoleate and linolenate were prepared by the urea fractionation of methyl esters from olive, safflower and linseed oils, respectively. Additional pure methyl linolenate was obtained from Nu-Chek-Prep, Inc. (Elysian, MN). Esters were purified according to Wong and Hammond (3) and oxidized in 5-g batches at 28 C unless otherwise specified. Peroxide values, iodometric reduction of the peroxides, separation of the methyl hydroxyesters, formation of the TMS-derivative, GLC and other analyses were performed as before (3).

The methyl hydroxyester from the oxidation of methyl linolenate was separated from those produced by oleate and linoleate by thin layer chromatography (TLC) on 0.5 mm thick plates containing 10% silver nitrate. The plates were developed in petroleum ether/diethyl ether (40:60, v/v) and bands were detected by spraying with 0.2% 2′,7′-dichlorofluorescein in ethanol and viewing under ultraviolet light. The methyl hydroxyesters were extracted from the silver ion plates by the Hill et al. procedure (8) and converted to TMS-derivatives as before (3).

Olive and soybean oils were randomized by stirring with 0.1% sodium methoxide at 60 C for 5 hr at 1 Torr. Randomized and natural oils were purified by chromatography through alumina before oxidation (9). After oxidation

---

[1] Journal Paper No. J-9657 of the Iowa Agriculture and Home Economics Experiment Station, Ames. Project 2143.

and reduction of the hydroperoxides, the oils were converted to methyl esters with 0.5% sodium methoxide in methanol and analyzed as before. Statistical analysis was according to SAS (10).

RESULTS AND DISCUSSION

The identity and purity of the TMS-derivatives of the methyl hydroxyesters produced from the oxidation of methyl oleate and linoleate have been established previously (3). To establish the identity of the TMS-derivative obtained from the oxidation of methyl linolenate, the hydroperoxides were reduced by iodide and the methyl hydroxyesters were isolated by TLC and silylated. GLC of the TMS-derivatives gave the 4 partly resolved peaks seen in Figure 1. These results are comparable to those reported by Frankel et al. (6) in their Figure 1B. An IR spectrum of the TMS-derivatives indicated the presence of TMS (845, 1250 cm^{-1}), methyl ester (1180, 1745 cm^{-1}), *cis,trans*-conjugated diene (950 cm^{-1}), and *trans,trans*-conjugated diene (988 cm^{-1}). The mass spectrum showed fragments at 183, 223, 311 and 351 and after hydrogenation at 131, 173, 187, 229, 259, 301, 315, 357 and 371 in agreement with the results of Frankel et al. (6).

GLC would not resolve the TMS-hydroxylinoleate and TMS-hydroxylinolenate, as a comparison of Figures 1 and 2 shows. Silver ion chromatography of the TMS-derivatives also failed to give satisfactory separation. Satisfactory resolution was obtained with methyl hydroxyesters on silver nitrate plates as shown in Figure 3. Only small amounts of hydroxyester (ca. 1 mg/spot) could be applied or resolution was lost.

Wong and Hammond (3) reported that the apparent yield by GLC of the TMS-derivatives was 63% from methyl oleate hydroperoxide and 40% from methyl linoleate hydroperoxide based on methyl heptadecanoate as an internal GLC standard. Pure methyl oleate, linoleate and linolenate were oxidized separately until they reached peroxide values of ca. 5, 10, 20 and 40 meq/kg. Samples were converted to TMS-derivatives, and GLC responses were measured compared with the response of methyl heptadecanoate. Results are in Figure 4. The apparent yields calculated from the slopes were 62, 44 and 17% for oleate, linoleate and linolenate hydroperoxide, respectively. Subsequent analyses were corrected by these factors. The reasons for these large correction factors are not certain. Wong and Hammond found that methyl hydroxystearate and methyl

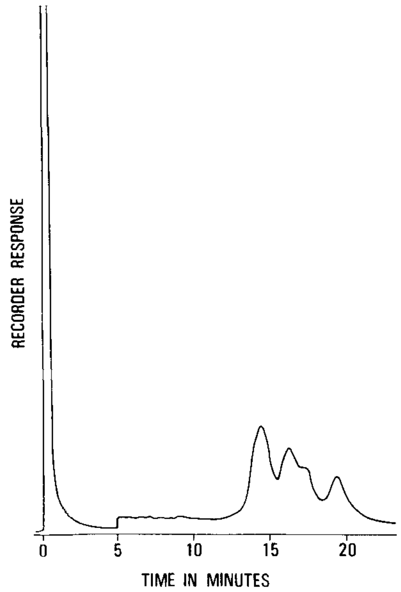

FIG. 1. A typical gas chromatogram of TMS-hydroxylinolenate on a 183-cm x .3-cm column packed with 10% OV 225 on Chromosorb W (HP). The carrier gas was nitrogen at 50 ml/min and the temperature was 180 C.

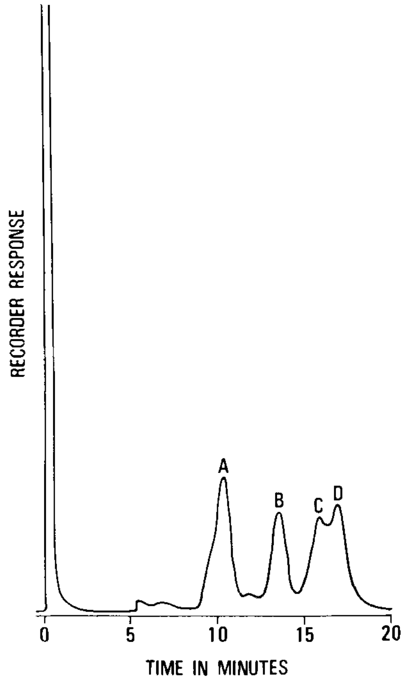

FIG. 2. A typical gas chromatogram of a mixture of TMS-hydroxyoleate and TMS-hydroxylinoleate. The conditions are the same as in Figure 1. Peak A is TMS-hydroxyoleate. Peaks B, C and D are TMS-hydroxylinoleate.

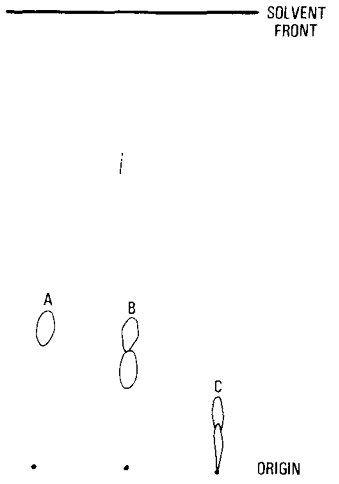

FIG. 3. TLC separation on silver nitrate-Silica Gel G of methyl hydroxyesters obtained from the reduction of hydroperoxides. The Silica Gel G contained 10% by weight of silver nitrate. The developing solvent was petroleum ether/diethyl ether (40:60, v/v). A is methyl hydroxyoleate, B methyl hydroxylinoleate and C methyl hydroxylinolenate.

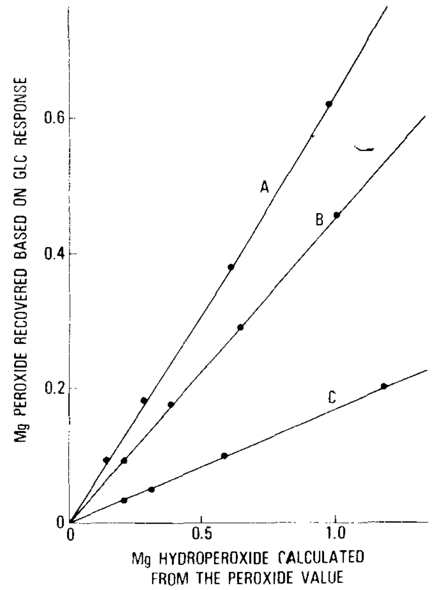

FIG. 4. The apparent recovery of TMS-hydroxy derivatives on the basis of GLC response compared with that calculated from the peroxide value. The slopes are a measure of the correction factors. A: methyl oleate; B: methyl linoleate; C: methyl linolenate.

ricinoleate both gave 78% yields and concluded that at least some of the correction factor was a result of the flame ionization detector's response to the silyl ethers. Some of the correction may arise from side reactions in the conversion of the hydroperoxides to TMS-hydroxyesters, but we have been unable to detect significant amounts of side products.

The analysis of the hydroperoxides in binary and trinary mixtures of the 3 esters is given in Table I. The data on oleate and linoleate are similar to those of Wong and Hammond (3), but include mixtures containing more oleate than they studied. In mixtures of 2 fatty acids the following propagation reactions can occur:

$R_1OO\cdot + R_1H \rightarrow R_1OOH + R_1\cdot$
with rate constant K_{11}
$R_1OO\cdot + R_2H \rightarrow R_1OOH + R_2\cdot$
with rate constant K_{12}
$R_2OO\cdot + R_2H \rightarrow R_2OOH + R_2\cdot$
with rate constant K_{22}
$R_2OO\cdot + R_1H \rightarrow R_2OOH + R_1\cdot$
with rate constant K_{21},

where R_1 refers to oleate and R_2 to linoleate. Fineman and Ross (11) have shown that

$$[(R_2OOH/R_1OOH) - 1]\,(R_1H/R_2H) = -(K_{11}/K_{12})\,(R_2OOH)/(R_1OOH)\,(R_1H/R_2H)^2 + K_{22}/K_{21}$$

and linear plots of the data allow K_{11}/K_{12} and K_{22}/K_{21} to be evaluated from slope and intercept. According to Russell (12), the various peroxy radicals are usually equally potent in abstracting hydrogen from RH and the rates of propagation are determined by the ease of abstraction of hydrogen from the various alkenes. If this is so $K_{11} \cong K_{21}$ and $K_{22} \cong K_{12}$ and $(K_{11}/K_{12})\,(K_{22}/K_{21}) \cong 1$. It follows also that, in trinary mixtures of oleate, linoleate and linolenate, data for oleate and linoleate should fall on the same line as the binary mixtures because it will be immaterial whether the peroxy radical is oleate, linoleate or linolenate, and all the hydroperoxide will accumulate according to the ease of hydrogen abstraction from the 3 alkenes. Such a plot for all the data in Table I is shown in Figure 5. The data from the trinary mixtures fall on about the same line as the binary data. $K_{22}/K_{21} = 8.38 \pm 0.54$ and $K_{11}/K_{12} = 0.0791 \pm 0.0070$. The product of the ratios is 0.66.

Not all of the points are equally reliable. When the amount of methyl oleate in the mixture is small, the amount of oleate hydroperoxide is very small and analytical error increases. This is particularly true because of a minor side product from linoleate oxidation that has the same retention time in the gas chromatograph as TMS–hydroxyoleate. This material is partly removed by TLC of the hydroxyesters, but when the TMS-hdyroxyoleate is small, this contaminant can cause significant error. In Figure 5, these are the points in which the percentage of oleate hydroperoxide was less than 10%, many of which fall well below the straight line plots. If we omit these values, the data are fit best by a line with K_{22}/K_{21} = 10.15 ± 0.66 and K_{11}/K_{12} = 0.0944 ± 0.0069. The product of the ratios is 0.96.

It is not certain, however, that all the deviation shown in Fig 5 is caused by experimental error. Frankel et al. (6) reported data for oleate-linoleate mixtures that also have relatively large proportions of oleate hydroperoxide in mixtures containing low proportions of methyl oleate. Their analysis, based on mass spectrometry, should not be subject to the error we encountered. Moreover, to omit the mixtures that contained less than 10% oleate hydroperoxide, we had to omit all mixtures containing less than 60% methyl oleate.

If the peroxy radicals are all essentially equal in their ability to extract hydrogen from the alkene chains, then the best estimate of the relative ease of abstraction of hydrogen from linoleate compared with oleate would be the average of K_{22}/K_{21} and K_{12}/K_{11} which is 10.37. This agrees with the average relative oxidation rates calculated in Table I from the oleate-linoleate mixtures with large proportions of methyl oleate.

A Fineman-Ross plot of the linoleate-linolenate data of Table I is given in Figure 6. The data for the binary and trinary mixtures again fall on a single line, K_{33}/K_{32} is 1.623 ± 0.098 and K_{22}/K_{23} is 0.3927 ± 0.0110, where 3 refers to linolenate. The product of the 2 ratios is 0.64. If we average K_{33}/K_{32} and K_{23}/K_{22}, we find that the relative ease of linolenate oxidation compared with linoleate is 2.08. This

TABLE I

GLC Analysis of the Formation of Peroxide Types in Autoxidized Oleate, Linoleate and Linolenate Mixtures

Composition of methyl ester mixture					Peroxide %			Relative oxidation rate[a]	
18:1	18:2	18:3	P.V.	Recovery %[b]	Monoene	Diene	Triene	18:2/18:1	18:3/18:2
87.1	12.9		25.3	92.1	38.15	61.84		10.94	
77.31	22.69		33.3	91.2	25.64	74.36		9.9	
68.51	31.49		34.4	86.04	19.57	80.43		8.95	
57.98	42.02		41.5	93.6	13.04	86.98		9.2	
38.65	61.35		47.4	88.5	8.42	91.57		6.04	
19.73	80.27		57.4	103.7	5.55	94.45		4.2	
	89.66	10.34	55.8	89.3		80.14	19.86		2.14
	69.77	30.23	41.9	90.4		51.73	48.27		2.15
	49.16	50.84	34.5	89.4		30.98	69.02		2.15
	17.97	82.03	44.2	102.3		9.52	90.48		2.08
89.37	5.54	5.09	42.3	94.3	34.54	21.82	43.64	10.2	1.83
89.33	7.31	3.35	44.2	102.1	36.62	32.39	30.99	10.8	2.08
89.23	3.75	7.02	41.2	96.7	35.23	14.77	50.00	9.97	1.8
79.48	10.42	10.2	56.8	91.9	23.3	29.13	47.57	9.54	1.7
79.38	13.79	6.83	40.3	94.5	22.98	41.92	35.09	10.5	1.7
			55.3	89.7	21.9	43.81	34.29	11.5	1.6
79.22	7.14	13.64	49.8	87.1	20.2	18.31	61.48	10.05	1.75
			59.9	85.9	18.48	19.18	62.33	11.5	1.7
69.17	15.54	15.29	45.2	90.8	13.92	27.84	58.23	8.9	2.1
			64.5	89.7	11.54	30.12	58.33	11.6	1.96
69.11	20.70	10.19	39.9	86.4	14.35	42.58	43.06	9.9	2.05
			69.7	87.7	12.10	44.37	43.57	12.2	2
69.23	10.63	20.14	8.6	91.5	13.14	17.81	69.05	8.8	2.05
			53.9	87.6	11.91	20.30	67.69	11.1	1.8
5.33	88.9	5.77	52.6	105.2	---	86.03	13.97	---	2.5
13.27	70.84	15.89	36.5	104.4	1.97	67.83	30.19	6.5	1.98
22.60	52.12	25.28	65.9	92.4	2.39	52.56	45.05	9.5	1.8
33.67	30.17	36.16	41.6	88.3	3.09	27.99	68.92	10.1	2.05
44.01	12.32	43.67	40.4	91.4	4.26	12.52	83.22	10.5	1.9
14.92	15.11	69.97	51.7	89.4	1.56	10.16	88.28	6.4	1.9
22.80	25.75	51.45	35.5	93.4	2.41	17.91	79.68	6.6	2.23
33.26	36.12	30.61	35.9	86.2	4.53	30.74	64.72	11.2	1.8
42.44	46.42	11.14	37.9	90.2	6.06	62.88	31.06	9.5	2.06

[a] % R_2OOH x % R_1H/% R_1OOH x % R_2H or % R_3OOH x % R_2H/% R_2OOH x % R_3H, where R_1 is oleate, R_2 linoleate and R_3 linolenate.

[b] By GLC after applying correction factors.

agrees with the relative oxidation rate of 2.09 calculated from Table I.

A plot of the oleate-linolenate data from the trinary mixtures of Table I is given in Figure 7. These data show considerably more scatter. For all the points, K_{33}/K_{31} is 16.503 ± 0.918 and K_{11}/K_{13} is 0.337 ± 0.0049. If the points representing low percentages of oleate hydroperoxide are omitted, the values are 17.357 ± 1.438 and 0.0368 ± 0.0061, respectively. The products of the sets of ratios are 0.56 and 0.64, respectively. The averages of K_{33}/K_{31} and K_{13}/K_{11} indicate the linolenate oxidizes 23.09 and 22.23 times faster than oleate. If we multiply the estimated rates for (linoleate/oleate) x (linolenate/linoleate), this should also give the linolenate/oleate rate and is 10.37 x 2.08 = 21.57.

For linoleate-linolenate and oleate-linolenate mixtures, the product of slope and intercept are

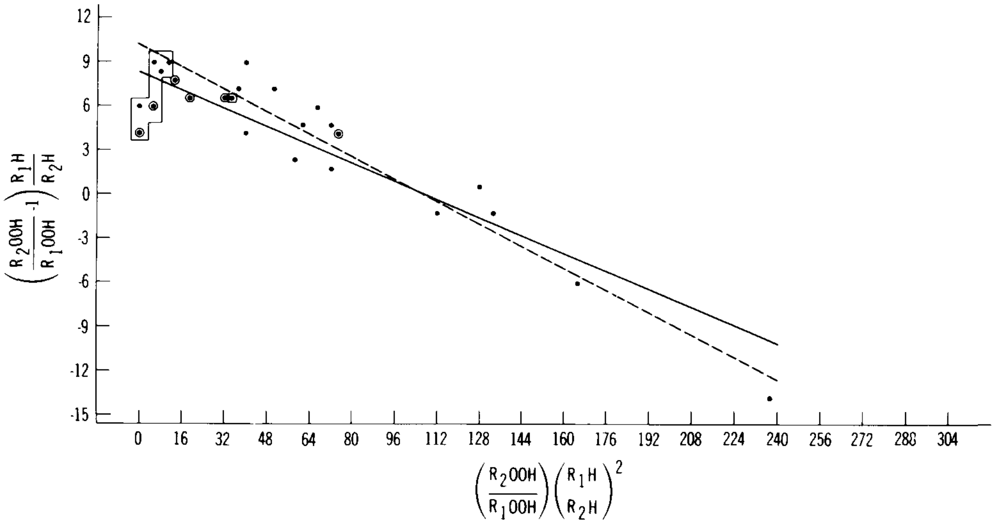

FIG. 5. A Fineman-Ross plot of the data for oleate and linoleate mixtures. The points with circles are from binary mixtures. The solid line is the best fit of all the points. The dashed line is the best fit of all points with more than 10% oleate hydroperoxide. The points with less than 10% oleate hydroperoxide are enclosed in rectangles. R_1 is oleate, R_2 linoleate.

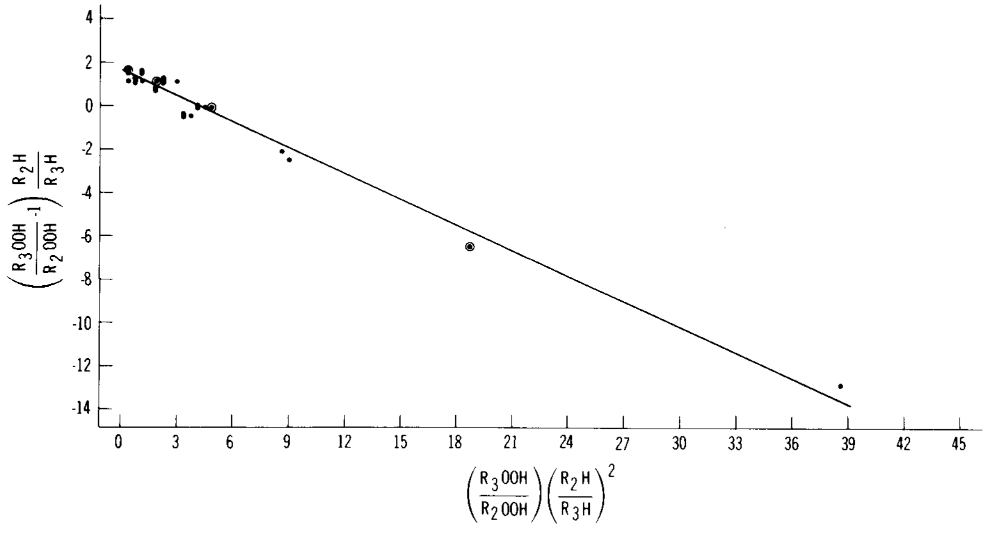

FIG. 6. A Fineman-Ross plot of the data for linoleate-linolenate mixtures. The circled points are from binary mixtures. R_2 is linoleate, R_3 linolenate.

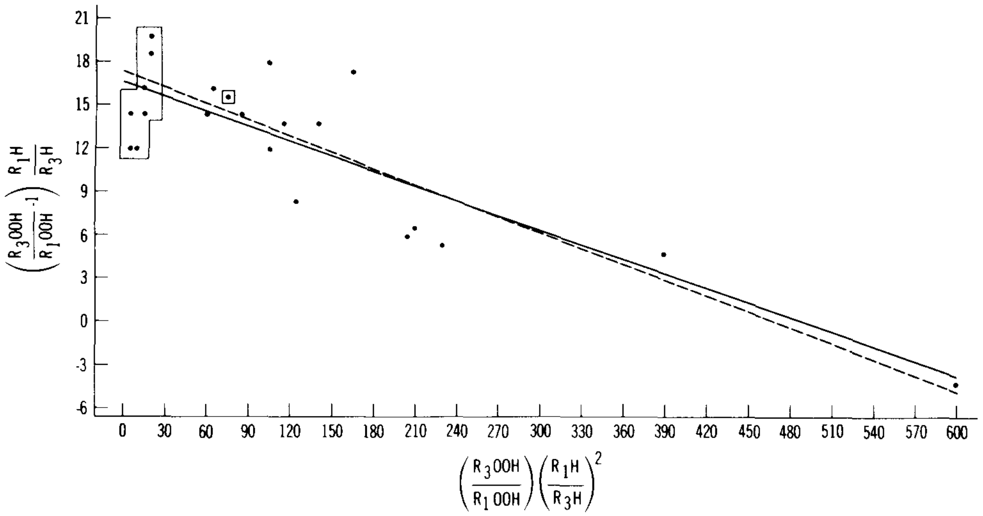

FIG. 7. A Fineman-Ross plot of the data for oleate-linolenate mixtures. The solid lines is the best fit for all the points with more than 10% oleate hydroperoxide. The points with less than 10% oleate hydroperoxide are enclosed in rectangles. R_1 is oleate, R_3 linolenate.

somewhat below 1. This may mean the peroxy radicals are not exactly equivalent or it may reflect some bias in the experimental data.

The relative oxidation rates we have found are very close to those obtained by the relative rates of oxidation of pure esters (1,2). This indicates that, in the oxidation of these pure esters, the rates of initiation must have been very similar. The relative rates of oxidation of these esters do not change appreiciably with extent of oxidation up to peroxide values of 70 meq/kg. Oxidations carried out at 21 C did not give results appreciably different from those at 28 C. The actual proportions of oleate and linoleate hydroperoxides we found are quite different from those reported by Frankel et al. (5,6), but this may be caused by the high temperatures and much higher peroxide values in their experiments. Such conditions are not conducive to quantitative recovery of hydroperoxides. Our results are much closer to those Frankel and Neff obtained for soybean oil (7), which were obtained under conditions more like the ones we used. But Frankel and Neff found in methyl ester mixtures prepared from soybean oil that at low peroxide values there was a relatively large proportion of 12-hydroxyoctadecanoate. The proportion of this isomer decreased as oxidation proceeded. We have noted no change with extent of oxidation in the proportions of products we isolated, but it is uncertain how a 12-hydroxydienoate or trienoate would behave in our separations.

Raghuveer and Hammond (13) suggested that glyceride structure can affect the relative rates of oxidation of acyl groups in a triglyceride. They suggested that acyl groups at the *sn*-1 and 3 positions should oxidize faster than those at *sn*-2. To study this, natural and randomized oils were oxidized and the analysis of the peroxides is given in Table II. The difference in the analysis of the randomized and unrandomized oils are the same within experimental error and similar to results expected on the basis of the methyl ester mixtures. However, this is not a very good test of Raghuveer and Hammond's theory. For example, we can calculate from the Fatemi and Hammond equations (14) that the unrandomized soybean oil used in this study should have an average composition on the *sn*-1, 3-positions of 26.60% oleate, 44.36% linoleate and 7.75% linolenate. Let us assume that the most extreme, and most easily detectible version of Raguhuveer and Hammond's proposal is that all the oxidation occurs on the *sn*-1, 3 positions and also that the relative rates of oxidation are those found here (1:10.3.21.6). Under these assumptions, the hydroperoxides should be found in the proportions 4.09% oleate, 70.20% linoleate and 25.72% linolenate. A similar calculation, assuming the soybean oil is completely randomized, yields values of 3.53%, 74.45% and 22.02%. The differences are not much greater than the experimental error of our method. Even so, the randomized oils oxidized about 3-4 times faster than the natural oils.

TABLE II

Analysis of the Hydroperoxides Formed during the Oxidation of Natural and Randomized Soybean and Olive Oils

Oil	% Fatty acid				P.V.	% Recovery	% Peroxide			Relative oxidative rate	
	Sat	18:1	18:2	18:3			Monoene	Diene	Triene	18:2/18:1	18:3/18:2
Soybean											
Natural	13.67	25.87	52.98	7.47	39.5	89.2	4.77	73.55	21.67	7.52	2.0
Random	13.61	25.44	53.24	7.71	36.1	87.4	4.57	72.87	22.54	7.7	2.13
Olive											
Natural	15.68	74.02	10.30	--	31.3	89.1	39.02	60.98	--	11.22	--
Random	16.20	73.16	10.64	--	40.6	91.6	40.50	59.50	--	10.09	--

REFERENCES

1. Gunstone, F.D., and T.P. Hilditch, J. Chem. Soc. 1945:836.
2. Holman, R.T., and O.C. Elmer, J. Am. Oil Chem. Soc. 24:127 (1947).
3. Wong, W.D., and E.G. Hammond, Lipids 12:475 (1977).
4. Frankel, E.N., W.E. Neff, W.K. Rohwedder, B.P.S. Khambay, R.F. Garwood and B.C.L. Weedon, Ibid. 12:901 (1977).
5. Frankel, E.N., W.E. Neff, W.K. Rohwedder, B.P.S. Khambay, R.F. Garwood and B.C.L. Weedon, Ibid. 12:908 (1977).
6. Frankel, E.N., W.E. Neff, W.K. Rohwedder, B.P.S. Khambay, R.F. Garwood and B.C.L. Weedon, Ibid. 12:1055 (1977).
7. Frankel, E.N., and W.E. Neff, Ibid. 14:39 (1979).
8. Hill, E.E., D.R. Husbands and W.E. Lands, J. Biol. Chem. 242:4440 (1968).
9. Jensen, R.G., T.A. Marks, J. Sampugna, J.G. Quinn and D.L. Carpenter, Lipids 1:451 (1966).
10. Barr, A.J., J.H. Goodnight, J.P. Sall and J.H. Helwig, "A User's Guide to SAS," SAS Institute Inc., Raleigh, NC, 1976.
11. Fineman, M., and S.D. Ross, J. Polym. Sci. 5:259 (1950).
12. Russell, G.A., J. Am. Chem. Soc. 77:4583 (1955).
13. Raghuveer, K.G., and E.G. Hammond, J. Am. Oil Chem. Soc. 44:239 (1967).
14. Fatemi, S.H., and E.G. Hammond, Lipids 12:1032 (1977).

[Received October 15, 1975]