

Formation of Peroxides in Fatty Esters. II. Methyl Linoleate: Application of the Polarographic and Direct Oxygen Methods

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IN a recent paper (6) the authors presented polarographic evidence showing that the major constituent formed during the initial stages of the autoxidation of methyl oleate at 80°C. is a hydroperoxide. In the methyl oleate study it was also observed that most of the oxygen absorbed in the initial stages of the autoxidation could be accounted for as hydroperoxide, whereas in the latter stages a large proportion of the absorbed oxygen was used to form other oxidized compounds.

Because of the encouraging results obtained by the use of the polarographic and direct oxygen methods in the methyl oleate study, the authors decided to investigate the autoxidation of methyl linoleate under identical experimental conditions and thus obtain data concerning the extent and type of peroxidic products formed.

As in the previous study, the modified Wheeler iodide (3) and the polarographic methods (5, 6) were used to determine the respective peroxide and hydroperoxide contents, and the Wijs iodine method was used to determine halogen uptake. Also included in the present study was the catalytic micro-hydrogenation method for determining the extent of unsaturation.

Apparatus and Materials

Polarographic Study. A Sargent Model XXI polarograph was used to obtain the current-voltage curves. The capillary had m and t values of 3.12 mg. per second and 1.53 seconds, respectively, yielding a capillary constant of 2.29 mg.^{2/3} sec.^{-1/2}. These values were obtained, using an open circuit with the capillary dipping into the electrolytic solution and the polarographic cell maintained at $25 \pm 0.1^\circ\text{C}$.

A modified H-type electrolytic cell previously described (5) was used; the electrolytic solution consisted of 0.3 M lithium chloride in 50-50 (by volume) absolute methanol-benzene. This cell, containing 40 ml. of the electrolytic solution, had a resistance of 1,175 ohms. All half-wave potentials were corrected for IR drop and were measured against a saturated calomel electrode.

Direct Oxygen Apparatus. The method used was essentially that described by Unterzaucher (4) except that the sample was pyrolyzed in a quartz combustion tube by an electric burner maintained at 1000°C. and that the nitrogen used to sweep the combustion products through the apparatus was freed of oxygen and moisture by passing it through a gas-purifying train.

Catalytic Micro-Hydrogenation Apparatus. The apparatus has been described by Ogg and Cooper (2) and is designed so that agitation is performed by magnetic stirring. The diminution of hydrogen volume is measured in a gas burette after adjusting the pressure with an adjacent manometer assembly.

Methyl Linoleate. The methyl esters of fatty acids from safflower oil were subjected to a series of low temperature fractional crystallizations from acetone and from petroleum ether in a manner similar to that described by Frankel *et al.* (1) in their preparation of linoleic acid from a number of vegetable oil sources.

The freshly prepared methyl linoleate had iodine value, 169.8 (Theory 172.4) and peroxide value, 27.5. After storage in a sealed vessel for several months and prior to its use in the oxidation experiments, the iodine value was 169.1, peroxide value, 93.

Procedure

Polarographic Analysis. The polarographic procedure has been described in previous studies (5, 6).

Direct Oxygen Determination. The direct oxygen determination has been described in a previous publication (6).

Catalytic Micro-Hydrogenation Determination. The hydrogenation of 15-25 mg. samples of autoxidized methyl linoleate dissolved in glacial acetic acid in the presence of approximately 20 mg. of palladium on carbon catalyst was performed as described by Ogg and Cooper (2). The volume of absorbed hydrogen, after correction to standard conditions of pressure and temperature, was expressed as hydrogen numbers (grams of sample per mole of hydrogen).

Chemical Peroxide Value. The method used was the Wheeler iodide method modified in that nitrogen flushing was used during the solution of the sample in the glacial acetic acid-chloroform solvent and the sample was in contact with the KI solution for 7 to 8 minutes in the dark (3).

Iodine Number. The iodine numbers were determined by the A.O.C.S. Wijs method.

Results and Discussion

Three polarographic waves for 0.2054, 0.3603, and 0.5291 g. per 40 ml. of electrolytic solution of an autoxidized methyl linoleate sample (140 hours at 80°C.) are shown in Figure 1 (Curves A, B, and C, respectively). The corrected half-wave potentials of these curves of -0.82, -0.80, and -0.79 volts, respectively, are within the half-wave potential range observed with pure hydroperoxides (-0.69 to -0.96 volts vs. S.C.E.) in previous studies (5, 6). Unlike the polarographic wave of methyl oleate hydroperoxide, these polarograms exhibit a preliminary wave at a half-wave potential of about -0.10 volts. It appears that some other peroxidic form is present with the methyl linoleate hydroperoxide, but the amounts present are too small to measure quantitatively.

From the first four columns of Table I, and from Figure 2, it can be seen that up to 174 hours of autoxidation, a point well past the peak chemical peroxide value, the polarographic values were consistently

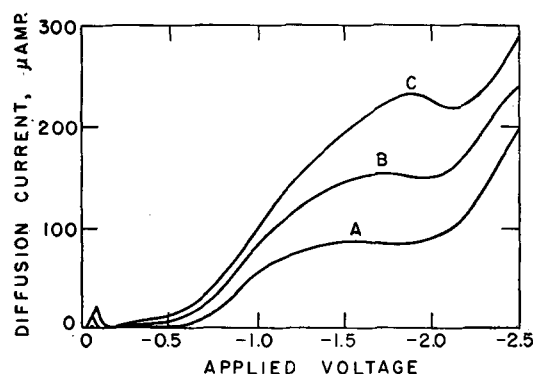


FIG. 1. Polarographic waves of three concentrations of methyl linoleate autoxidized 140 hours at 80°C.

lower, the average differences being 12.7%. After the peak value, the chemical values decreased faster so that toward the end of the autoxidation the polarographic method gave the higher peroxide values. This discrepancy between polarographic and chemical peroxide values had been observed in a previous study in which methyl oleate was autoxidized at 80°C. (6). Figure 3 shows plots of the relative percentage differences between the values obtained by the two methods for samples of methyl oleate (A) and methyl linoleate (B), both autoxidized at 80°C., plotted against hours of autoxidation. These plots show that up to the peak value, in the case of methyl linoleate (B), the polarographic peroxide values are more than 10% lower than the chemical peroxide values whereas up to the peak value for methyl oleate (A), the differences between the two methods bracket zero and average 3.3%. A further point of difference between the methyl oleate and methyl linoleate autoxidations is that, whereas the polarographic peroxide values for methyl oleate become less than the chemical peroxide values during the latter stages of the autoxidation, the reverse is true in the case of methyl linoleate. In contrast, it has been observed that with high purity

Tetralin, cumene, methyl oleate, and methyl linoleate hydroperoxides the polarographic and chemical peroxide values agreed within $\pm 2\%$.

The higher chemical peroxide values found up to 174 hours may have occurred because peroxides other than hydroperoxides were formed during autoxidation which were non-reducible polarographically but which reduced chemically. It is more difficult to explain why the polarographic peroxide values were higher than the chemical values toward the end of the autoxidation period. Perhaps these high polarographic values may be attributed to the formation of

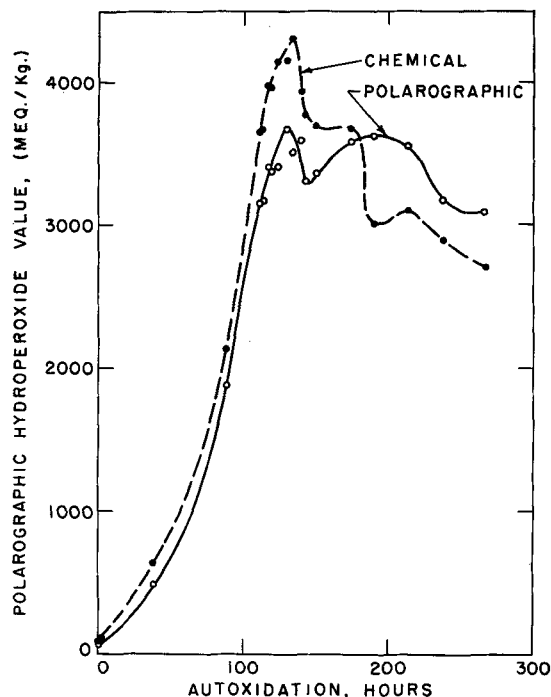


FIG. 2. Comparison of polarographic and chemical peroxide values for methyl linoleate autoxidized at 80°C.

TABLE I
Analytical Data Obtained for Samples of Methyl Linoleate Autoxidized from 0 to 267 hours at 80°C.

Hours of autoxidation	Polarographic peroxide values	Chemical peroxide values	Relative % difference	Total oxygen, %	Oxygen other than ester, %	Oxygen as hydroperoxide, %	Residual ^a oxygen, %	Free acid, meq./g.	Hydrogen numbers	Corrected hydrogen numbers expressed as iodine values ^b	Iodine numbers
0	78	93	-16.1	0.00	149.3	169.0	169.0
2	111	117	-5.1	11.54	0.75	0.18	0.57	0.00	145.1	173.5	168.8
38	488	632	-22.8	11.93	1.19	0.78	0.41	0.00	145.3	169.0	166.0
88	1883	2137	-11.9	14.39	3.95	3.01	0.88	0.02	142.1	154.7	149.4
111	3147	3650	-13.8	18.06	8.07	5.03	2.83	0.07	150.3	128.4	124.8
113	3172	3720	-14.7	18.09	8.10	5.07	2.82	0.07	151.2	126.7	121.8
117	3399	3984	-14.7	18.15	8.16	5.44	2.46	0.08	157.2	118.3	115.2
119	3366	3960	-11.8	19.18	9.32	5.39	3.58	0.11	162.5	111.3	111.5
124	3403	4144	-17.9	20.49	10.79	5.44	5.00	0.11	187.3	89.6	103.5
130	3674	4150	-11.5	21.60	12.04	5.88	5.68	0.15	181.1	90.9	93.7
134	3496	4300	-18.7	21.54	11.97	5.59	5.80	0.18	185.8	89.4	87.4
140	3583	3925	-8.7	22.90	13.95	5.73	7.32	0.28	173.5	98.8	80.3
143	3297	3769	-12.5	23.51	14.19	5.27	7.93	0.31	187.6	102.7	80.3
150	3325	3686	-9.8	23.48	14.15	5.32	7.45	0.43	225.2	89.6	72.2
174	3567	3668	-2.8	24.97	15.82	5.71	8.13	0.62	231.4	66.2	57.0
190	3621	3000	+20.7	25.64	16.57	5.79	8.22	0.80	243.5	63.1	49.8
214	3553	3100	+12.8	25.63	16.56	5.68	8.19	0.84	265.5	59.2	42.2
238	3174	2886	+10.0	26.55	17.59	5.08	9.44	0.96	277.4	52.3	36.4
267	3086	2700	+14.3	26.84	17.92	4.94	9.56	1.07	321.4	39.8	31.4

^a Oxygen other than ester, hydroperoxide or free acid.

^b Hydrogen numbers corrected for polarographic hydroperoxide content by the following equations:

- (1)
$$\frac{2 \times 10^6}{\left\{ \frac{I}{H \text{ No.}} \times 2 \times 10^6 \right\} - \text{peroxide value}} = \text{corrected hydrogen number}$$
- (2)
$$\frac{253.8}{\text{Corrected H No.}} \times 100 = \text{hydrogen number expressed as iodine number}$$

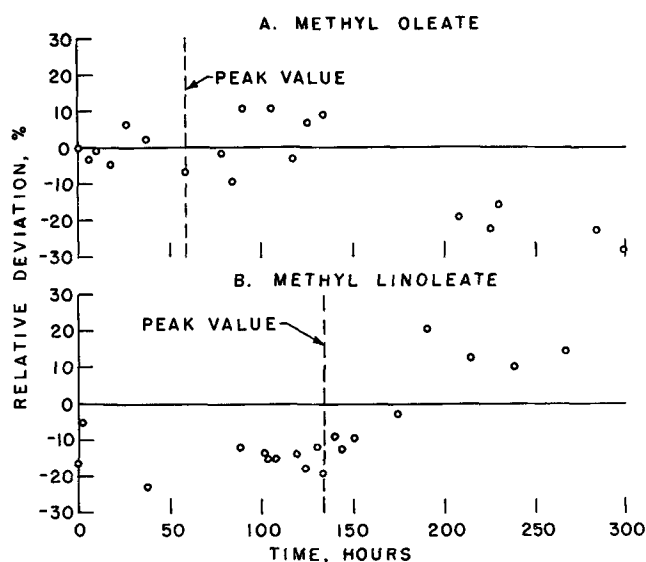


FIG. 3. Relative percentage differences between polarographic and chemical peroxide values obtained for methyl oleate (A) and methyl linoleate (B) autoxidized at 80°C.

diketotype compounds which had been observed to have polarographic characteristics similar to those of the hydroperoxides (half-wave potentials of -0.80 volts) (5). If present, these compounds would increase the polarographic and not the chemical peroxide values.

Another cause of the higher peroxide values obtained by the polarographic method during the latter stages of autoxidation may be due to the presence of peroxides which reacted slowly in the chemical method, that is, liberated iodine only after long standing. We have recently observed that with some concentrated solutions of cyclohexene hydroperoxide in cyclohexene (98-100%), the chemical peroxide approached the polarographic peroxide values only after 3 hours of standing in contact with the potassium iodide solution. This would indicate that small amounts of a peroxide may be present which liberate iodine at a much slower rate from the iodide solution than that observed for the hydroperoxide.

The curves of Figure 2 also show that after oxidizing methyl linoleate for 267 hours at 80°C. an appreciable amount of hydroperoxide, 62.8% of the peak chemical value and 84.0% of the peak polarographic value, was still present. By comparison, when methyl oleate had been oxidized for the same length of time at 80°C., there remained only 8.5 and 7.4% of the chemical and polarographic peak values, respectively (6).

As was expected, methyl linoleate attained a peak hydroperoxide value of about 4,000 whereas methyl oleate under the same experimental conditions reached a maximum value of only 2,000 (6). In the case of methyl oleate the maximum value was reached in 59 hours whereas it required more than twice this time for methyl linoleate to reach its peak value. Thus the rate of hydroperoxide build-up was similar for both materials. The hydrogen numbers, iodine numbers, oxygen, and free acid contents for the samples of methyl linoleate at various stages of autoxidation are shown in Table I. This table shows that the observed hydrogen numbers, column 10, decreased during the first 88 hours of autoxidation, indicating that the

accumulation of hydroperoxide was more rapid than the destruction of double bonds. After this point the reverse is indicated. The continuous decrease in iodine numbers however would indicate that double bonds were being destroyed throughout the autoxidation. As shown in the fifth column of this table, the total oxygen content at the end of 267 hours was 2.33 times as much as that present at 2 hours of autoxidation. The free acid content, column 9, rose slowly during the first half of the autoxidation, reaching a value of only 0.18 meq./g. at the end of 130 hours. During the latter stages the free acid content increased progressively faster to a final value of 1.40 meq./g., indicating that chain scission occurred mostly in these latter stages.

Data presented in columns 5-8 show how the oxygen content was distributed between different functional groups. Included are values for total oxygen, total oxygen corrected for ester oxygen, oxygen as hydroperoxide, and the residual oxygen in functional groups other than ester hydroperoxide, or acid. These data, plotted against hours of autoxidation, are shown in Figure 4, where Curve A is the total oxygen other

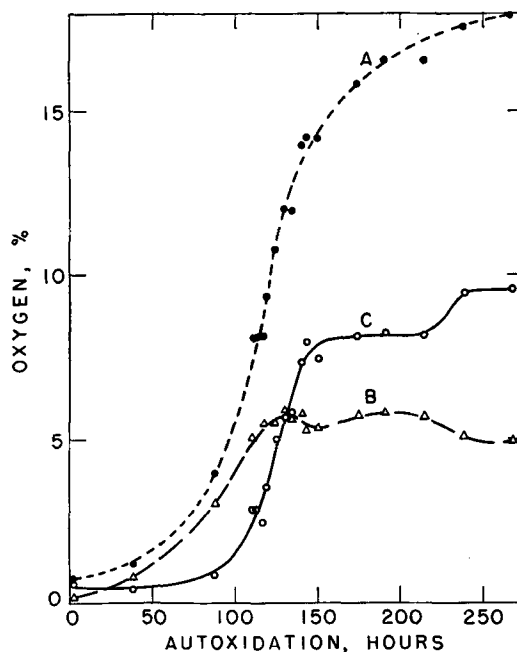


FIG. 4. Distribution of absorbed oxygen in methyl linoleate autoxidized at 80°C.

- A. Total oxygen other than ester oxygen.
- B. Oxygen present as hydroperoxide.
- C. Oxygen other than hydroperoxide, ester, or acid.

than ester, Curve B is the oxygen present as hydroperoxide, and Curve C is the oxygen present in functional groups other than ester, hydroperoxide, or acid. Curve A of this figure shows that most of the oxygen uptake in the initial stages of autoxidation can be accounted for as hydroperoxide (Curve B). However after 100 hours of autoxidation a considerable quantity of the oxygen uptake is being used to form oxygenated compounds other than hydroperoxide. The levelling off of Curve A after 180 hours shows that the autoxidation process has slowed down considerably. In Curve C is shown the oxygen present throughout the autoxidation which cannot be accounted for either as ester, hydroperoxide, or as free acid. It

will be noticed that there is little unaccounted for oxygen in the system until after 90 hours of autoxidation. Then a rapid rise occurs until 8% of the material is oxygen in forms other than those groups mentioned above. It is curious that the unaccounted for oxygen curve parallels the hydroperoxide curve very closely from 160 to 212 hours. Presumably the unaccounted for oxygen is in the form of carbonyl or hydroxyl groups which were not measured by any of the methods used in this study.

The observed hydrogen numbers (obtained by the catalytic microhydrogenation method) and the halogen uptake, as shown by the iodine numbers, are shown in Table I, columns 10 to 12, respectively. It

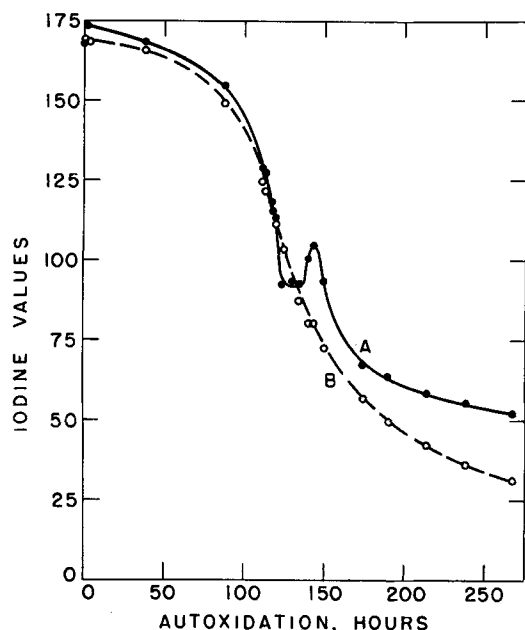


FIG. 5. Extent of unsaturation of methyl linoleate autoxidized at 80°C. as shown by the catalytic hydrogenation and Wijs iodine methods.

A. Corrected hydrogen numbers expressed as iodine numbers.
B. Wijs iodine numbers.

can be seen from the data that the halogen uptake (iodine numbers) decreases with the extent of autoxidation. Since it is difficult to compare iodine values (grams iodine per 100-g. sample) with hydrogen numbers (grams of sample per mole of hydrogen) the latter have been calculated on the basis of iodine values, and, in addition, the hydrogen numbers have been corrected for hydroperoxide content on the basis of the respective polarographic values, column 11.

In Figure 5 are shown the iodine values (Curve B) and the corrected hydrogen numbers (Curve A) plotted against the hours of autoxidation. The two curves show good agreement up to 118 hours of autoxidation, but, from this point up to 150 hours, the hydrogen numbers show erratic behavior while the iodine values continue smoothly downward. The divergence of the curves after about 130 hours of autoxidation may be attributed to the accumulation of conjugated products which are known to give incomplete iodine absorption. No attempt has been made to draw any quantitative conclusions from either the iodine or hydrogen numbers. These data on highly oxidized materials are probably only of qualitative significance.

Summary

This study of the prolonged autoxidation of methyl linoleate at 80°C. has included polarographic identification and determination of hydroperoxides, the direct determination of oxygen contents, and catalytic micro-hydrogenation for the determination of unsaturation. The polarographic method has further substantiated the observations of other workers that the principal peroxidic substance formed during the autoxidation of methyl linoleate at 80°C. is a hydroperoxide. The direct oxygen measurements have shown that most of the oxygen absorbed in the initial stages of autoxidation can be accounted for as hydroperoxide. During the latter stages there was a continuous increase of oxygen uptake, half of which can be accounted for as free acid and half as forms other than hydroperoxide, ester, or free acid. By means of the catalytic micro-hydrogenation method it has further been shown that as the autoxidation progresses there is a continuous decrease in unsaturation.

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