Formation of Peroxides in Fatty Esters. I. Methyl Oleate. Application of the Polarographic and Direct Oxygen Methods[1]

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THE identity of the peroxides that form during the autoxidation of fatty esters has long been the subject of intensive investigation. Farmer and Sutton (1), by means of continuous molecular distillation, succeeded in isolating a peroxide from a sample of autoxidized methyl oleate. They found that their peroxide fraction consisted mostly of the unsaturated monohydroperoxide, a small amount of the dihydroperoxide, together with some peroxide decomposition product. The study of autoxidation peroxidic products would be facilitated were an analytical method available for the identification and quantitative estimation of the peroxides, applicable to autoxidation mixtures without the necessity of preliminary separations.

Lewis and Quackenbush (2) found that autoxidized methyl linoleate and autoxidized lard dissolved in a nonaqueous electrolytic solution produced well-defined polarographic reduction waves. They suspected that these waves were due to the peroxides present in the autoxidized samples because the polarographic wave heights were proportional to the respective chemical peroxide values. They did not however identify the peroxidic compounds responsible for these waves.

The present authors have developed a polarographic method which is not only qualitative but also is quantitative for the determination of peroxides and hydroperoxides. This method provides a means for the direct measurement of these substances in autoxidation mixtures (7). Their polarographic method not only distinguishes between peroxides and hydroperoxides, but the polarographic waves of these compounds in autoxidation mixtures are directly proportional to concentration. The precision of the polarographic method for measuring hydroperoxides has recently been established by a statistical comparison of the results obtained by this method with those of two chemical methods (4).

In the present study the authors have applied this polarographic method to autoxidized methyl oleate to follow the peroxide formation. Other analyses included are the Unterzaucher method for the measurement of oxygen (5), the Wheeler iodide method (3) for peroxides, and the Wijs iodine method for halogen uptake.

Apparatus and Materials

Polarographic Study. A Sargent Model XXI polarograph was used to obtain current-voltage curves. The capillary had m and t values of 4.63 mg. per second and 1.47 seconds, respectively, yielding a capillary constant of 2.96 mg.$^{2/3}$ sec.$^{-1/2}$. The curves were obtained by using open circuit with the capillary dipping into the electrolytic solution and the polarographic cell maintained at 25 ± 0.1°C.

A modified H-type electrolytic cell previously described (7) 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 and showed an average increase of only 5 ohms when 2 grams of methyl oleate were added. All half-wave potentials were corrected for IR drop and measured against a saturated calomel electrode.

Direct Oxygen Apparatus. The sample was pyrolized in a quartz combustion tube by an electric sample burner maintained at 1,000°C. This burner consisted of a coil of nickel-chromium resistance wire having an inside diameter slightly larger than the diameter of the combustion tube and a stainless steel shell with a heat-reflecting aluminum lining. The long furnace similar to that described by Walton *et al.* (6) was maintained at 1,120 ± 5°C. 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.

Methyl Oleate, I, for Stability Study. Methyl oleate with an initial chemical peroxide value of 35.3 was heated at 80 ± 1°C. under an atmosphere of nitrogen. The head space above the methyl oleate was flushed periodically with nitrogen to insure an inert atmosphere. Samples were withdrawn at 0, 0.2, 2, 4, and 16 hours for polarographic and chemical peroxide determinations.

Methyl Oleate, II, for Prolonged Autoxidation Study. Methyl oleate with an initial chemical peroxide value of 7.7 was heated at 80 ± 1°C. for 296 hours with oxygen bubbling through it at the rate of 1 ml. per second. Samples were removed at intervals for determination of peroxide values by both polarographic and chemical methods, for iodine numbers and direct oxygen analysis.

Procedure

These two studies were made a) to determine the stability of peroxides stored at 80°C. in an inert atmosphere and b) to follow the formation and degradation of peroxides in methyl oleate oxygenated at 80°C. from 0 to 296 hours. Samples were withdrawn from the reaction flasks at intervals predetermined by trial runs under identical conditions so that samples could be taken at those times of greatest changes in peroxide content. The samples were analyzed for peroxide content by both the polarographic and chemical peroxide methods. In addition, Wijs iodine numbers and total oxygen contents were determined for methyl oleate II. Replicate samples for check analyses were stored at −20°C.

Polarographic Analysis. The polarographic procedure was essentially that described in previous studies (4, 7), wherein polarograms of samples of autoxi-

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dized methyl oleate (0.2000 to 0.5000 g.) were obtained using the 0.3 M lithium chloride, methanol-benzene electrolytic solution. Pure methyl oleate does not reduce polarographically under these experimental conditions. The presence of hydroperoxides in autoxidized methyl oleate was shown by the polarographic waves having half-wave potentials of about —0.80 volts vs. the saturated calomel electrode. Polarograms were obtained at three different concentration levels for each sample and from these the hydroperoxide contents, in terms of peroxide values, were calculated from the diffusion currents by the following equation:

$$\text{Polarographic Peroxide Value} = \frac{i_d}{KC} \times 2000$$

The derivation and description of this equation will be given under "Results and Discussion."

Direct Oxygen Determination. Five to 10 mg. samples of the autoxidized methyl oleate, weighed in a micro platinum boat, were placed in the combustion tube, the system was purged of oxygen by back-sweeping with nitrogen, and the sample was pyrolized at 1,000°C. The carbon monoxide formed when the oxygen from the sample passed through the heated carbon filling (1,120°C.) was dried by passing over solid potassium hydroxide and subsequently reacted with iodine pentoxide at 113°C. The liberated iodine was trapped in 20% potassium hydroxide solution and oxidized to the iodate, and the iodine liberated by the addition of potassium iodide was titrated with 0.02 N sodium thiosulfate.

Chemical Peroxide Value. Samples of methyl oleate, 0.2 to 0.4 gm., were analyzed by the modified Wheeler iodide (3) method. The weighed samples were transferred to a 250-ml. Erlenmeyer flask. The flasks flushed with a stream of nitrogen while adding 20 ml. of the solvent (60 parts of glacial acetic acid and 40 parts of chloroform), the sample dissolved by swirling, and one ml. of saturated KI added. After the stoppered flasks had stood in the dark for 7 to 8 minutes, 50 ml. of distilled water were added and the liberated iodine was titrated with 0.1 N thiosulfate solution. Starch was used as the indicator. The peroxide value, P.V. (milliequivalents of peroxide oxygen per kg.), was calculated by the following equation:

$$\text{P.V.} = \frac{\text{ml.} \times \text{N} \times 1000}{\text{S}}$$

where S is the weight of sample and N the normality of the thiosulfate solution.

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

Polarographic Behavior of Autoxidized Methyl Oleate

Curve II, Figure 1, shows the polarographic curve for autoxidized methyl oleate. This wave had a corrected half-wave potential of —0.80 volts vs. the saturated calomel electrode. The authors had observed in a previous study (7) that of all the pure oxygen-containing organic compounds examined only the hydroperoxides and the diketostearic acids reduced at or near this half-wave potential. The diketostearic acids were eliminated as the possible autoxidation products because these compounds could

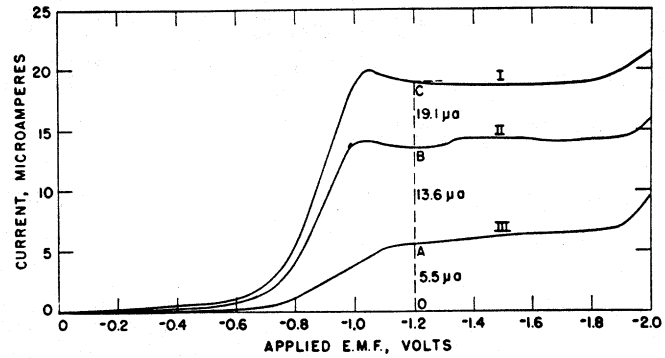

FIG. 1. Polarogram of autoxidized methyl oleate and p-menthane hydroperoxide.

I. 4.0×10^{-4} M p-menthane hydroperoxide + autoxidized methyl oleate (0.5142 grams/40 ml.).

II. Autoxidized methyl oleate (0.5142 grams/40 ml.).

III. 4.0×10^{-4} M p-menthane hydroperoxide.

not be reduced by a chemical peroxide method, whereas the autoxidation products of methyl oleate were reducible by both the chemical and the polarographic methods.

To confirm that the autoxidation product of methyl oleate, which had a reducible wave at —0.80 volts, was a hydroperoxide, known amounts of p-menthane hydroperoxide were added to the electrolytic solution containing a known amount of the autoxidized methyl oleate. Curve III, Figure 1, shows the polarographic reduction wave for a sample of p-menthane hydroperoxide (4.0×10^{-4} M) having a diffusion current of 5.5 microamperes (OA). Curve II shows the polarographic wave for autoxidized methyl oleate (0.5142 g. per 40 ml., chemical peroxide value = 106) having a diffusion current of 13.6 microamperes (OB), and Curve I shows the wave for a solution containing the same concentrations of autoxidized methyl oleate and of p-menthane hydroperoxide used to obtain Curves B and A, respectively. All these polarographic waves not only have the same half-wave potential, but the solution of the mixture has a diffusion current of 19.1 microamperes (OC), which is the sum of the diffusion currents obtained for the two components. This shows that the diffusion currents are additive and that the half-wave potentials of autoxidized methyl oleate and p-menthane hydroperoxide are identical, giving strong evidence that the polarographically reducible peroxidic substance in the autoxidized methyl oleate is a hydroperoxide.

Calculation of Polarographic Peroxide Values

It had been observed (7) that the diffusion current consists of organic hydroperoxides of diverse molecular structure including 96.5% pure methyl oleate hydroperoxide had essentially the same diffusion current constants, 5.85 ± 0.05; therefore accurate determinations of the hydroperoxide content of autoxidized methyl oleates are possible. From the diffusion current for a known concentration of autoxidized methyl oleate and from the diffusion current of a known concentration of pure hydroperoxide obtained with the same capillary, it was possible to calculate the polarographic peroxide value expressed in milliequivalents of peroxide oxygen per kg. of sample by substitution in the following equation:

$$\frac{i_d}{KC} \times 2000 = \text{Peroxide Value} = \text{milliequivalents of peroxide oxygen per kg. of sample}$$

where i_d = diffusion current in microamperes for sample of autoxidized methyl oleate.

K = diffusion current in microamperes/millimole/liter for a standard hydroperoxide using the same capillary.

C = concentration of autoxidized methyl oleate in H-cell expressed as grams per liter.

Results and Discussion

Analysis of the samples withdrawn at intervals from methyl oleate I, heated under nitrogen at 80°C., gave the values shown in Table I. It is to be noted from the data that the polarographic peroxide values are lower than the chemical values up to 4 hours of heating. The polarograms of these samples showed that the only polarographically reducible substance present was a hydroperoxide. The data also indicate that even though the autoxidized methyl oleate had been heated under nitrogen at 80°C. for 16 hours, both methods showed the presence of reducible peroxides. However, because of the low peroxide level, the differences shown in the peroxide values by the two methods may not be significant.

TABLE I

Chemical and Polarographic Peroxide Values. Sample 1. Methyl Oleate (Original Chemical Peroxide Value = 35.3) Heated at 80°C. under Nitrogen

Hours of Heating	Peroxide Values	
	Chemical	Polarographic
0.0	35.3	27.5
2.0	39.5	33.3
4.0	38.2	38.4
16.0	37.5	35.6

Methyl oleate II, initial peroxide value of 7.7, was autoxidized for 296 hours to follow the formation and fate of the peroxidic products throughout the course of autoxidation. Table II shows that the peroxide values reached a peak of over 2,000 at the end of 59 hours of autoxidation and that thereafter the peroxide values decreased until at the end of 296 hours they were 142 and 102 for the chemical and polarographic methods, respectively.

TABLE II

Peroxide Values of Methyl Oleate Autoxidized at 80°C.

Hours of autoxidation	Chemical peroxide value	Polarographic peroxide value	Relative % difference
0	7	7	0.00
6	252	245	−3.17
10	519	515	−0.77
19	971	931	−4.12
27	1311	1396	+6.48
37	1586	1614	+2.14
59	2133	2002	−6.14
79	1850	1818	−1.73
84	1693	1532	−9.51
91	1431	1581	+10.48
106	1041	1114	+10.70
117	920	895	−2.72
126	732	784	+7.10
134	623	681	+9.31
208	229	187	−18.34
226	209	163	−22.01
250	197	166	−15.74
283	167	130	−22.16
296	142	102	−28.17

It is to be noted that the greatest relative percentage of difference between the chemical and polarographic values occurred when the peroxide values decreased to less than 600. Data which have been obtained on other samples of autoxidized methyl oleate, not a part of this study, and which had peroxide values up to 1,000 have shown that the relative percentage of difference between the chemical and polarographic peroxide values may be as much as 28%. The polarographic values are the lower. The lower polarographic values may be due either to the fact that the hydroperoxide originally formed had been transformed to some peroxidic material not reducible polarographically but which was reducible by the non-specific chemical method, or that other peroxides or hydroperoxides were formed which influenced the chemical values.

The authors recognize that considerable difference of opinions exist among oil chemists as to whether or not the iodine values of autoxidized materials necessarily give a true measure of the double bonds. However since it is quite customary to report iodine values, they have been included in this study and these data are given in Figure 2 (Curve D). This curve shows that the iodine values decreased rapidly during the first 120 hours of autoxidation and that after this point they remained constant with iodine values of only 2.9 and 2.7 at the end of 226 and 296 hours, respectively.

Table III and Figure 2 show the distribution of the oxygen consumed at various stages of autoxidation. Curve A in Figure 2 is the oxygen present as hydroperoxide calculated from the polarographic data, and it also represents the changes in hydroperoxide content with time; curve B is the total non-ester oxygen obtained by subtracting the ester oxygen from the direct oxygen data; and curve C is the oxygen present in forms other than ester and hydroperoxide.

Table III shows that the oxygen not accounted for as ester or hydroperoxide was only 0.52% at the start but that this unaccounted-for-oxygen increased steadily during the course of the autoxidation. Of the 26.14% oxygen found in the methyl oleate at the end of the autoxidation, more than half (15.21%) existed in forms other than ester or hydroperoxide oxygen.

TABLE III

Direct Oxygen Determination of Methyl Oleate Autoxidized at 80 ± 1°C.

Hours of autoxidation	% Oxygen found	% Hydroperoxide (polarographic) found	% Hydroperoxide oxygen [a]	% Non-ester oxygen [a]	% Oxygen other than ester and hydroperoxide [a]
10	12.03	8.46	0.82	1.34	0.52
27	13.74	22.93	2.23	3.19	0.96
59	16.74	32.88	3.20	6.30	3.10
79	19.18	29.86	2.91	8.71	5.80
91	20.17	25.97	2.53	9.66	7.13
106	20.91	18.30	1.78	10.31	8.53
134	22.16	11.19	1.09	11.49	10.40
208	24.12	3.07	0.30	13.36	13.06
226	24.53	2.68	0.26	13.77	13.51
250	24.99	2.73	0.27	14.22	13.95
283	25.42	2.14	0.21	14.65	14.44
296	26.14	1.68	0.16	15.37	15.21

[a] Calculated values.

Summary

The polarographic method presented here for the quantitative determination of hydroperoxides is sim-

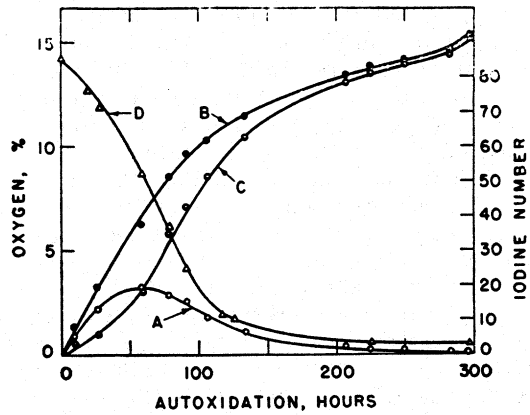

FIG. 2. Changes in oxygen content and iodine number of methyl oleate during autoxidation. (A = hydroperoxide oxygen, B = total non-ester oxygen, C = oxygen other than ester and hydroperoxide, and D = iodine number.)

ple, rapid, and applicable to the analysis of materials containing a mixture of different peroxidic groups. The application of this nonaqueous polarographic technique to the study of the changes occurring during the prolonged autoxidation of methyl oleate at 80°C. has permitted the identification and quantitative estimation of the hydroperoxide formed during this process. The selectivity of the polarographic method makes it possible to identify and measure the hydroperoxide even when present with other peroxidic forms. A comparison of the data obtained by this new technique and by the chemical peroxide method has been made and shows that the polarographic method for hydroperoxides is specific and quantitative.

By means of a direct oxygen method it has been possible to follow accurately the change in the oxygen content during the autoxidation. These data have confirmed the work of other investigators using other methods, in that the oxygen absorbed in the initial stages is used primarily in hydroperoxide formation, whereas in the latter stages the oxygen is consumed to form non-hydroperoxidic materials. The methods used in this study should be of great value in studying the changes which occur in drying oils for these changes are induced by autoxidation.

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