

Polarographic Studies of Fat Oxidation¹

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EVER SINCE the importance of peroxides in the autoxidation of fats was recognized, attempts have been made to identify and study the different peroxides involved. For lack of better methods, most of the evidence was based on analogy with simpler processes and on the analysis of breakdown products. During the autoxidation of asymmetric diphenylethylene Staudinger (1) showed that two peroxides of differing stability were formed. By analogy and from variations in the different analytical constants of oxidized fats, formation of several types of peroxides in oxidized fats was inferred. Farmer and co-workers (2) were the first actually to isolate and identify a peroxide, that is, the hydroperoxide. Since then methyl oleate hydroperoxide has been isolated in purer form, and it is now generally accepted that the hydroperoxide is one of the first and major products of autoxidation of unsaturated fatty acids. Even after the isolation of the hydroperoxide, there was no method available for detecting and estimating hydroperoxide in the presence of other peroxides.

Lewis and Quackenbush (3), using a polarograph for the study of autoxidized fat, obtained three waves which they showed were due to peroxides and which were not parts of the same wave. They did not however identify the peroxides responsible for the three waves. They used a mixture of methyl alcohol and benzene as solvent and lithium chloride as supporting electrolyte. Willits and co-workers (4) with the same solvent-electrolyte combination studied polarograms of a large number of oxygen-containing organic compounds having the same functional groups as were believed to occur in autoxidized fats. They observed that the acyl peroxides were reduced in the range 0–0.2 volts, and 2-diketones and hydroperoxides between 0.6–1.0 volts, and the unsaturated aldehydes and ketones around 1.5 volts and beyond; methyl oleate hydroperoxide was found to give a wave around 0.8 volt.

The present work involved a study of the polarographic behavior of peroxides formed in fats during autoxidation at 60°C. in the presence and absence of a catalyst.

Experimental

The autoxidations were conducted at 60°, by bubbling dry air through the fat or methyl esters and exposing to a 275-watt sunlamp. The polarograms were carried out in a 0.3 M solution of lithium chloride in a solvent consisting of equal proportions of methyl alcohol and benzene. The sample size varied from 0.01 g./100 ml. to 0.40 g./100 ml., chosen so as to give complete solution. The polarograms were obtained on a Sargent model XXI recording polarograph, using an H-type cell maintained at 30°. A saturated calomel electrode was used as reference electrode. The capillary used had a drop time of 1.5 seconds and mercury flow rate of 4.71 mgms./sec. The cell had a resistance of approximately 1,000 ohms, and the polarograph was used at a sensitivity of 0.04 microamperes per millimeter.

The peroxide value, expressed as milli equivalents per kilogram was determined on 0.1–0.5 g. of the sample, using a reaction time of 15 min. The solubility was tested by determining whether 0.2 ml. of the sample in 10 ml. Skellysolve F gave a clear or turbid solution.

The methyl esters used for comparison with soybean oil were prepared from fatty acids obtained by saponification of soybean oil. The methyl esters were distilled before use and had an iodine value of 127.3. The soybean oil used was a commercial, refined, and deodorized sample with an iodine value of 133.1.

Results and Discussion

As a first step in this investigation soybean oil was autoxidized at different temperatures. In the autoxidation at 0° and room temperature the samples were soluble in Skellysolve F even after oxidation for 20 days and showed a small wave at 0.2 volts and a prominent wave around 0.7 volts. The sample oxidized at 60° was however sufficiently polymerized at

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the end of a week to give an insoluble fraction in Skellysolve F. The polarogram of this sample showed the presence of a large wave at 0.2 volts in addition to other waves at more negative potentials. A sample of ethyl linoleate oxidized under similar conditions gave only a small wave at 0.2 volts; the major one was the hydroperoxide wave at 0.8 volts.

This prompted a comparative study of autoxidations of methyl esters and triglycerides of similar composition. The autoxidized samples showed three definite waves, named A, B, and C. The heights of these waves were measured at 0.3, 1.4, and 1.8 volts, respectively. For the autoxidized methyl esters of soybean fatty acids the height of different waves produced by samples varying in peroxide value is shown in Table I. The height of the wave is expressed as

TABLE I
Autoxidation of Methyl Esters of Soybean Fatty Acids
($m \frac{2}{3} t \frac{1}{6} = 3.02$)

Time Hours	Peroxide Value Milli-equivalents per kilogram	Height of Waves Diffusion Current/gm. Micro-amperes			Solubility in Skellysolve F
		A	B	C	
0	30	0.0	0.8	0.1	Soluble
17	2025	3.9	114.1	12	Insoluble
65	1085	2.0	71.5	13	Insoluble
95	840	1.3	51.3	14.4	Insoluble

micro-amperes per 1% (W/V) solution of the sample. The solubility refers to solubility in Skellysolve F. The peroxide giving rise to the A-wave could not be identified, and for convenience we shall call it peroxide-A. The B-wave was apparently due to hydroperoxides (4), and the C-wave was probably due to unsaturated aldehydes and ketones-decomposition products formed during autoxidation. The values of A and B rise and fall in the same way as the peroxide value. The height of C became constant.

The corresponding data for soybean oil autoxidation are shown in Table II. The rise and decline of

TABLE II
Autoxidation of Soybean Oil

Time Hours	Peroxide Value Milli-equivalents per kilogram	Height of Waves Diffusion Current/gm. Micro-amperes			Solubility in Skellysolve F
		A	B	C	
0.0	2.0	0.0	0.0	Soluble
12.5	109	0.8	2.6	Soluble
23	391	3.7	9.0	Soluble
36.5	2395	24	71.5	Insoluble
65.5	1455	12.6	48.0	Insoluble

A and B and the peroxide value were similar to those in methyl esters; C was not noted because of maxima in B. The value of A was much higher and that of B, the hydroperoxide wave, much lower than in methyl esters of comparable peroxide value.

The polarograms of the methyl esters and triglycerides are given in Figure 1. The polarogram of the methyl esters (I) showed a small wave at 0.2 volts, followed by a large wave due to hydroperoxide and then another short wave. The polarogram of the soybean oil sample (II) had a much larger A-wave than (I). The B-wave, that is, the hydroperoxide wave, was not as prominent as for the methyl esters. In the earlier stages of autoxidation the hydroperoxide wave

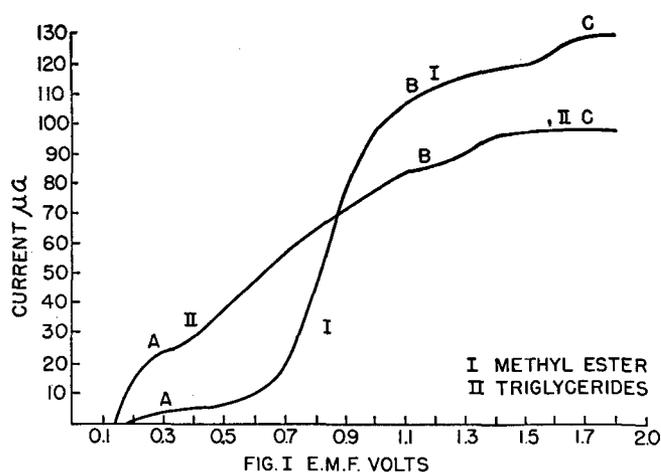


FIG. 1. Polarograms of samples of autoxidized methyl esters of soybean fatty acids and soybean oil. Methyl esters P.V. 2025; triglycerides P.V. 2395.

was well defined, but as polymerization set in, it was less and less sharp and ultimately gave an almost straight line increase from the A-wave up to about 1.4 volts. Although the peroxide value of the soybean oil sample was greater, the total diffusion current was less.

A comparison of the data from the methyl esters and the soybean oil is given in Table III. The ratio

TABLE III
Comparison of Methyl Esters and Soybean Oil Autoxidation

Methyl Esters			Soybean Oil		
Peroxide Value	B/A	P.V./A.	Peroxide Value	B/A	P.V./A.
.....	109	3.3	136
.....	391	2.4	105
2025	29	520	2395	3.0	100
1085	36	542	1455	3.8	115
840	39	645

of B to A indicated the extent of formation of peroxide A in comparison with the hydroperoxide, and the ratio of peroxide value to A indicated the extent of formation of peroxide A in comparison with total peroxides. These ratios do not actually represent the ratio of the concentration of the respective peroxides but are only proportional to them. The possibility of A and B being parts of the same wave was discounted because oxidation at 0° and room temperature gave very small A-waves unlike that at 60°. Also hydroperoxides in methyl esters do not give the same B/A ratio. Pure methyl oleate hydroperoxide does not give the A-wave at all. That the ratio B to A was distinctly lower in the case of soybean oil showed that peroxide A was formed in much greater proportions relative to hydroperoxides in the fat than in the methyl esters. The lower ratio of peroxide value to A showed that this was true in the case of total peroxides also.

The diffusion current constant depends on the diffusion coefficient of the molecules. Therefore it would be expected that a hydroperoxide group in a triglyceride would have a lower diffusion current constant, and hence for the same peroxide value the wave produced by a triglyceride hydroperoxide would be smaller than that in the case of methyl esters. The equal

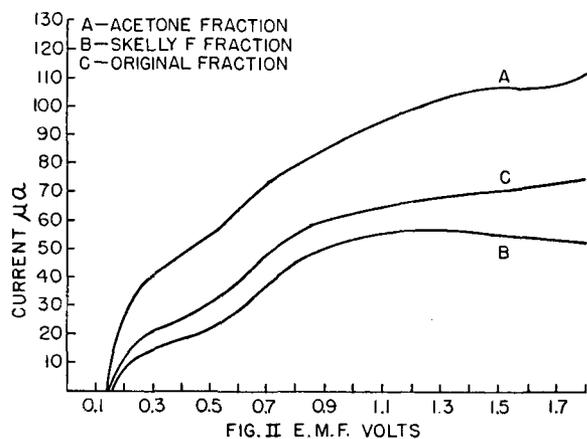


FIG. 2. Polarograms of fractions of autoxidized soybean oil.

peroxide values will ensure that the same number of peroxide groups per unit weight are present in each sample. This should be true for peroxide A also. In spite of this fact the height of the A-wave was considerably larger, thus showing again that peroxide A was formed in much greater proportions in soybean oil as compared to methyl esters of similar mixed fatty acid composition.

Attempts were made to study the formation of peroxides, particularly peroxide A, in the initial stages of autoxidation. Since solubility of triglycerides in the polarographic solvent was limited, extraction of peroxides from a larger weight of fat was tried. Privett and co-workers (5) used a method which involved extraction of peroxides by aqueous ethanol saturated with Skellysolve F. In the present study therefore the same solvent was used for extracting any peroxides present in fat. Lithium chloride supporting electrolyte was dissolved in this extract, and the polarogram was studied. As the hydroperoxide wave in this solvent was found to occur at much more positive potentials (starting from 0.14v), this method had to be abandoned.

A sample of soybean oil was oxidized at 60° to a peroxide value of 1530 milliequivalent per kilogram. Twenty g. of this oil were fractionated, using Skellysolve F containing different proportions of acetone. In the initial stages of polymerization the fat was soluble in small proportions of Skellysolve F, but on dilution the polymers precipitated out. Twenty g. of the sample were dissolved in Skellysolve F, and the solution was diluted to 300 ml. The portion separating out was allowed to settle and then separated by decantation. A similar procedure was followed to separate fractions, using Skellysolve F with acetone. The last fraction was dissolved in pure acetone. After fractionation the solvents were removed under vacuum at room temperature, and polarograms of the fractions were studied, using methyl-alcohol benzene solvent.

The polarograms of the original sample, the Skellysolve fraction, and the acetone fraction are shown in Figure 2. The hydroperoxide wave, particularly in the acetone fraction, was not well defined. This difficulty also occurred in all highly polymerized samples. The first wave height of the acetone fraction, as measured at 0.3 volts, was 40 micro-amps./g., that is, double that in the original sample or about two and a half times that in Skellysolve fraction.

Hydrogenation of the polymerized oil reduced all peroxides as the polarogram of the hydrogenated oil did not show any waves. But reoxidation of this sample produced the wave at 0.2 volts and a gradual rise up to 1.4 volts. A sample of soybean oil hydrogenated similarly and, to about the same extent (I.V 78), gave well-defined waves at 0.2 and 0.8 volts. Soybean oil samples hydrogenated to different iodine values and an olive oil sample all gave well defined A-waves and hydroperoxide waves. In all cases when the height of the A-wave was in the neighborhood of 10 micro-amps./g., 0.2 ml. of the sample showed turbidity in 10 ml. of Skellysolve F.

To study further the different types of peroxides, oxidation of the oils was carried out in the presence of a catalyst. As catalysts could affect the rate of formation or decomposition of certain types of peroxides more than others, catalysts could conceivably be used to get one peroxide in greater concentration than is normally formed. With this idea in mind oxidation of soybean oil in the presence of 0.1% cobalt-drier was studied. It was observed that the peroxide value did not reach a high level. But even at the maximum level reached (P.V. 474) the polarograms showed no waves due to the peroxide A or the hydroperoxide.

The polarograms of samples of soybean oil oxidized with and without cobalt catalyst are shown in Figure 3. While the sample oxidized without catalyst showed

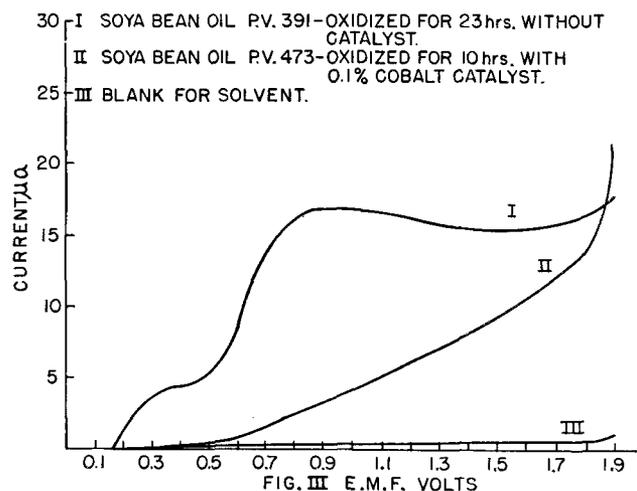


FIG. 3. Polarograms of samples of soybean oil autoxidized in absence and presence of a catalyst.

two clear waves, the sample with 0.1% cobalt showed a gradual increase up to the hydrogen wave. This suggested that the peroxides formed in the presence of a drier are different from those formed in the absence of a catalyst.

In the autoxidation of methyl oleate Swern and co-workers (6) found up to 28% peroxide other than hydroperoxide, which was not reduced at the dropping mercury electrode under the conditions used for the estimation of the hydroperoxide. They believed this peroxide to be the cyclic peroxide, as in several cases the alpha-glycol content of the reduced peroxides tallied with the difference between the total and the hydroperoxide. Knight and co-workers (7) have isolated 9-10 dihydroxystearic acid by oxidation of methyl oleate in acetic acid in the presence of cobalt

catalyst. Skellon and Taylor (8) isolated 8% dihydroxybehenic acid by oxidation of brassidic acid in presence of uranium catalyst. This suggested that the peroxide value of soybean oil autoxidized in the presence of cobalt was due to cyclic peroxide. Methyl oleate autoxidized similarly in the presence of cobalt also did not give the hydroperoxide wave. However attempts to concentrate the peroxides from this sample by the solvent extraction method and by the urea complex method were not successful.

Summary

The polarograms of autoxidized soybean oil indicated the presence of a peroxide, which was formed in considerably larger proportion in soybean oil than in methyl esters of similar composition. This peroxide was reduced at more positive potentials than the hydroperoxide at the dropping mercury electrode. In solvent fractionation, using mixtures of Skellysolve F and acetone, this peroxide associates with the poly-

merized fraction. Hydrogenated soybean oil samples and olive oil also showed the presence of this peroxide in considerable concentrations in the period that they began to give turbid solutions in Skellysolve F. Soybean oil oxidized in the presence of cobalt catalyst contained negligible proportions of peroxides normally reduced at the dropping mercury electrode. Attempts to concentrate the peroxides from methyl oleate oxidized in a similar manner did not succeed.

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