approximately 30 grams each, and each aliquot was analyzed for oil and nitrogen content. Figure 4 shows the random deviations from the mean in percentages of oil and nitrogen of the individual samplings.

Variations in these groups of analyses are much smaller than in the two-variety mixture samples, and deviations may be largely accounted for by variations in chemical analyses. There seems to be no accurate means of separating chemical errors from sampling differences in this case. Generally, the inverse correlation is quite high between oil and nitrogen content of a group of samples which differ appreciably in nitrogen and oil. If we assume that this is true for small variations in aliquots from a single lot of beans, we may use this correlation coefficient as a measure of agreement between oil and nitrogen analyses. The coefficient of correlation between oil and nitrogen analyses was —.377 for the Lincoln variety and —.244 for the Mandarin variety. This low correlation seems to indicate no appreciable error due to sampling difference when small lots of carefully selected soybeans of a single variety are sampled.

Conclusion

Under the conditions of this study, 30-gram samplings of mixtures of soybeans were found to differ significantly in oil and nitrogen content indicating the desirability of larger samples. The use of 120 to 240-gram aliquots from mixtures of soybeans which vary widely in chemical composition should tend to reduce differences due to sampling to a reasonable minimum. Differences among 30-gram samplings of highly uniform soybean seed of a single variety seem to be of slight significance. The limitations of present sampling methods should be recognized in any comparison or interpretation of chemical analyses of soybean seed.

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Changes Occurring in Fat Autoxidation[1, 2]

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Introduction

THE FACT that a rancid fat has a lower mean unsaturation and mean molecular weight, and lesser amounts of unsaturated fatty acids than a freshly refined fat has been known for some time. The impression seems to prevail that these characteristics cannot be used as criteria of the extent of oxidative rancidity. The basis for this impression is not readily apparent in the absence of rate studies on the decrease in iodine value, saponification equivalent, and the disappearance of specific unsaturated fatty acids. Therefore, it seemed desirable to study the rates of change of peroxide and iodine values, saponification equivalents, and the amounts of "linoleic and linolenic acids" during accelerated rancidification of a fat. An attempt has been made in the present investigation to correlate and interpret the experimental data in terms of existing theories concerning autoxidative deterioration.

Experimental

The substrates examined were commercial edible fats and oils purchased on the local market. Two of the samples were hydrogenated vegetable oil shortenings and the third was a refined cottonseed oil. The fatty acid compositions were such that the percentage of octadecadienoic acids ranged from 10 to 55% of the total fatty acids.

Method of Oxidation

The oxidation was carried out under the conditions of the modified Swift stability test (1). A large number of samples was oxidized in order to follow the chemical changes occurring over a relatively long period of time.

If an antioxidant or synergist was incorporated into the substrate, precautions were taken to secure a uniform dispersion. Samples were removed from the heated oil bath at definite time intervals and refrigerated until the entire series of samples from one experiment was available for analyses. The cooled samples were warmed until fluid and well agitated before weighed aliquots were removed for chemical analysis.

Analytical Constants

(a) Peroxide values (P.V.) were determined by a modified Wheeler method (2) and were expressed as milliequivalents of sodium thiosulfate per kilogram of fat.

(b) Iodine values (I.V.) were determined by the approved A.O.A.C. method, using Wijs reagent for a 30-minute reaction time.

(c) Saponification equivalents (S.E.) were determined by a modification of the approved A.O.A.C. method.

Spectrophotometric Determination of Unsaturated Fatty Acids

The amounts of diene and triene conjugated material originally present in the fat samples or induced in them during the course of the oxidation were determined spectrophotometrically in purified isooctane. The amount of diene and triene material present was calculated, using the $E^{1\%}_{1\,\mathrm{cm.}}$ values (corrected to methyl esters) previously accepted and used

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The suggestions of Dr. B. F. Daubert in the preparation of this manuscript are gratefully acknowledged.

[2] Presented at the 35th Annual Meeting of the American Oil Chemists' Society, May 10 to 12, 1944, New Orleans, La.

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by Bradley and Richardson (3) for 9:11 linoleic acid and *β*-eleostearic acid.

Total octadecadienoic and octadecatrienoic acids, calculated as the methyl esters, were measured by a modification (4) of the spectrophotometric method of Mitchell, Kraybill, and Zscheile (5).

The proportion of oleic acid (as methyl oleate) was calculated from the iodine value of the sample, and the amounts of dienoic and trienoic acids (as methyl esters) determined spectrophotometrically. Inasmuch as the spectrophotometric method gives maximum values for dienoic and trienoic acids, the oleic acid concentration as calculated may be consistently low by a small amount.

Discussion

The Effect of Oxidative Rancidity on the Rate of Peroxide Formation, Rate of Change of Iodine Value, and Rate of Change of Saponification Equivalent

The rate of formation of organic peroxides during the oxidation was found to follow a curve typical of an autocatalytic reaction (Figure 1). The flat portion of the curves prior to the rapid rise in peroxide concentration is a measure of the induction period (i.e., stability) of a fat or oil. In keeping with the investigations of Bailey, *et al.*, (6, 7) the cottonseed oils were found to be much less stable than the hydrogenated shortenings.

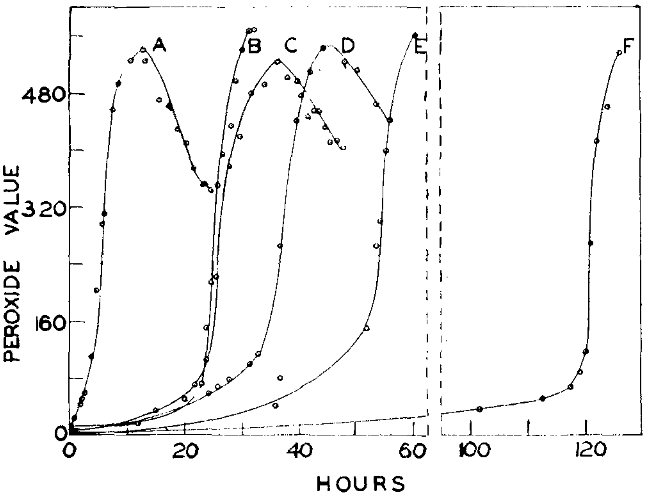

FIG. 1. The rate of peroxide formation in edible fats and oils with and without added antioxidant or synergist.

A. Cottonseed oil
B. Cottonseed oil + gallic acid
C. Shortening A
D. Shortening B
E. Shortening B + ascorbyl palmitate
F. Shortening B + gallic acid

The peroxide concentration on prolonged heating reached a maximum value and then decreased. This observation had previously been reported by Wheeler (2, 8) and Freyer (9), and appears to be due to thermal decomposition of the peroxides formed during the oxidation.

The effect of an added antioxidant or synergist is observed in the prolonged induction periods of the various fortified substrates. For all substrates, with or without added antioxidant or synergist, the rate of peroxide formation after the induction period was the same. The relative constancy of these rates is evident from the parallel slopes of the curves as shown in Figure 1.

The rate curve for the decrease in iodine value (Figure 2) is similar to that for the change in peroxide value. The iodine value remained relatively constant during the induction period but decreased rapidly immediately following it. For each system examined, the break in the rate curve for iodine value coincided with that for peroxide value. It has been shown by Hamilton and Olcott (10) that organic peroxides do not interfere with the determination of iodine values. Thus, the results obtained may be considered valid. The presence of an added antioxidant or synergist did not affect the general nature of the curve, but served only to increase the length of the induction period.

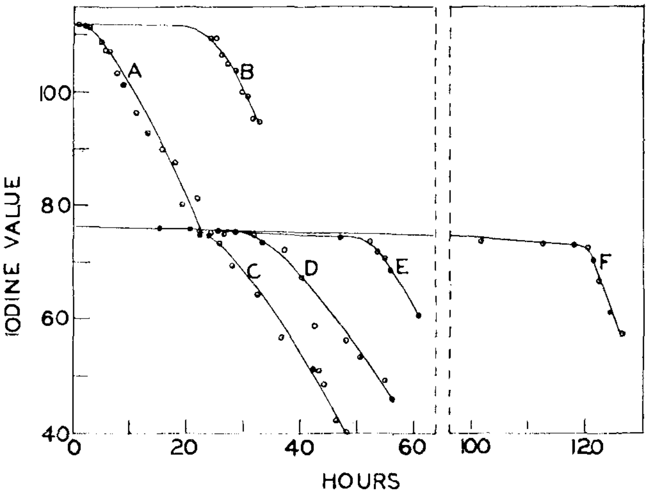

FIG. 2. Decrease in iodine value during fat autoxidation.

A. Cottonseed oil
B. Cottonseed oil + gallic acid
C. Shortening A
D. Shortening B
E. Shortening B + ascorbyl palmitate
F. Shortening B + gallic acid

It was found that the saponification equivalent of a fat decreased during the progress of oxidative rancidity. Scission of the carbon chain at the point of unsaturation must have occurred, resulting in molecules of lower mean molecular weight. Again, the rate curves (Figure 3) are typical of autocatalytic reactions. The curves are not as regular as the others, due

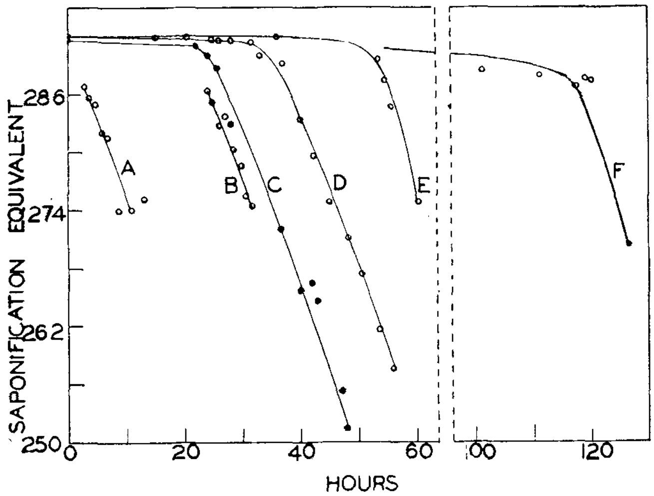

FIG. 3. Decrease in mean molecular weight of glycerides during fat autoxidation.

A. Cottonseed oil
B. Cottonseed oil + gallic acid
C. Shortening A
D. Shortening B
E. Shortening B + ascorbyl palmitate
F. Shortening B + gallic acid

to the errors involved in the saponification equivalent determinations made on the oxidized fats.

Effect of Oxidative Rancidity Upon Unsaturated Fatty Acids

From the ultra-violet absorption studies of the heated substrates it was apparent that diene conjugated material was formed during the oxidation (Figures 4 and 5). Enhanced ultra-violet light absorption at 230 mμ was indicative of the formation of new conjugated diene systems. Although the characteristic peaks indicative of triene conjugated material at 270 mμ and 280 mμ disappeared in the course of the oxidation, the general absorption in this region increased.

Bradley and Richardson (11) observed that this phenomenon occurred in linseed oil heated *in vacuo* at 575° F. The disappearance of the characteristic triene peaks and the marked general absorption in the region of 270 mμ to 280 mμ was explained by these authors as being due to cyclic dimers which have general absorption properties in this region.

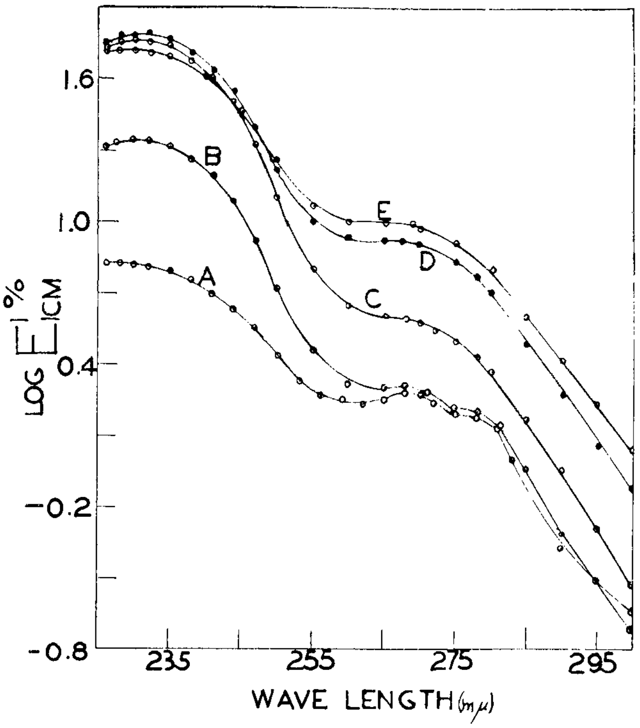

FIG. 4. Absorption curves of oxidized cottonseed oil.

A. Unoxidized oil		(P.V. = 12)
B. Oxidized for	3¾ hrs.	(P.V. = 132)
C. Oxidized for	7¾ hrs.	(P.V. = 457)
D. Oxidized for	13½ hrs.	(P.V. = 526)*
E. Oxidized for	20½ hrs.	(P.V. = 411)

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* The maximum peroxide value (541) was reached at 13 hours.

As the oxidation of either a hydrogenated vegetable oil shortening or cottonseed oil proceeded, the diene concentration reached a maximum and then decreased. Curves D and C in Figures 4 and 5, respectively, apparently represent an equilibrium between the rate of formation of conjugated diene material and its destruction by oxidation or polymerization.

Mitchell and Kraybill (12) reported the formation of diene conjugated material in linseed oil bodied *in vacuo* at 585° F. or steam blown at 230° F. while Overholt and Elm (13) indicated the formation of conjugated systems in drying paint films. These latter studies were not based on spectrophotometric methods but rather on the maleic anhydride reaction. A correlation was also made between the degree of peroxidation and the amount of diene conjugated substances. Recently Brauer and Steadman (14) studied the autoxidation of a highly purified sample of β-eleostearic acid. Spectrophotometric measurements of the oxidizing substrate indicated that new conjugated diene systems were formed while the triene material present decreased in concentration with time.

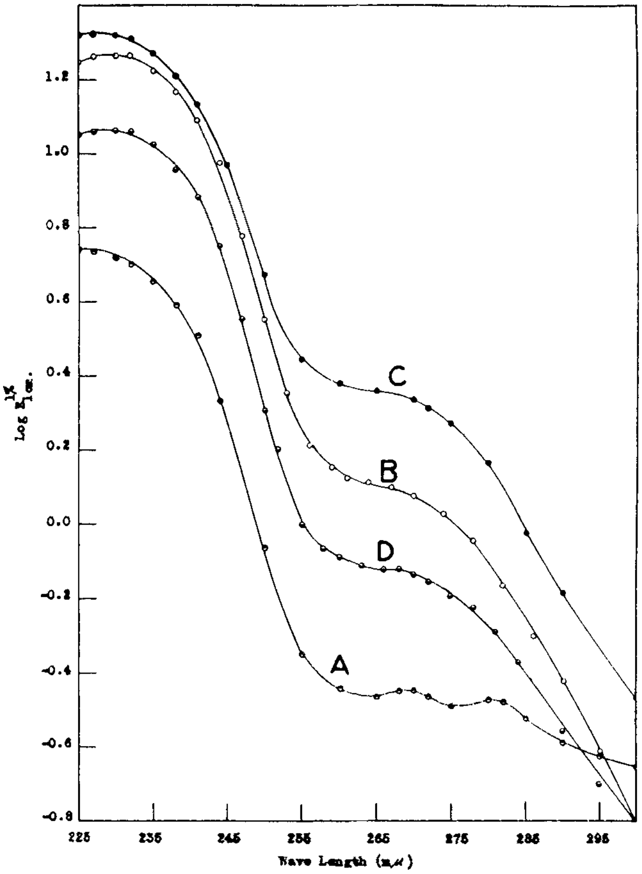

FIG. 5. Absorption curves of oxidized shortening B.

A. Unoxidized sample	(P.V. = 1.3)
B. Oxidized for 36 hrs. at 110°	(P.V. = 265)
C. Oxidized for 40 hrs. at 110°	(P.V. = 440)
D. Oxidized for 54 hrs. at 110°	(P.V. = 461)*

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* The maximum peroxide value reached in this experiment was 541 at 45 hours.

Farmer, *et al.*, (15) demonstrated that the ability of a double bond system to conjugate was dependent on the nature of the methylenic linkage. Thus linolenic acid and fish liver oils, which possess structures capable of resonance, can form conjugated systems while rubber and squalene, whose structures differ, cannot conjugate. The resonating compound is an olefinic free radical formed during the first stages of peroxidation by the severance through the action of molecular oxygen of a thermally activated α-methylenic C-H bond.

Approximately 5% of the total diene material initially present in the hydrogenated vegetable shortenings investigated was conjugated. During the course of the oxidation the total diene material decreased, but the preformed conjugated diene material increased. As much as 60% of the total diene material was found to be conjugated in an oxidized sample. In the case of cottonseed oil the initial diene concentration was high, but only 1% of the total diene

TABLE 1

Data on Ultra-Violet Studies of Oxidized Substrates

Sample	Hours aerated	Peroxide value	Total octadecadienoic acids as methyl esters %	Total octadecatrienoic acids as methyl esters %	Preformed conjugated octadecadienoic acids as methyl esters %	Preformed octadecatrienoic acids as methyl esters %	% of the total diene material present as preformed conjugated
Shortening A	0	2	10.2	0.4	0.6	0.02	6.4
	22	71	10.0	0.3	1.0	0.03	10.0
	25.5	223	9.1	0.6	2.3	0.09	24.7
	36.5	512	3.6	1.8	1.8	0.16	48.6
	44	455	2.4	2.3	1.7	0.19	69.6
	47	413	2.1	2.6	1.4	0.12	67.6
Shortening B	0	2	10.2	0.2	0.5	0.02	4.5
	33	70	9.5	0.5	1.5	0.18	16.2
	37	265	8.2	0.9	1.6	0.07	19.8
	40	440	6.2	1.2	1.8	0.13	29.3
	45	541	3.5	1.2	1.7	0.14	48.9
	54	461	2.3	1.6	1.0	0.05	42.2
Cottonseed Oil	0	12	55.4	0.3	0.6	0.11	1.1
	3.75	132	53.8	0.4	1.9	0.13	3.5
	7.75	457	47.7	1.4	5.1	0.24	10.6
	13.5	526	38.5	2.3	5.3	0.49	13.7
	20.5	411	28.4	2.8	4.7	0.57	16.4

material was conjugated. With increased oxidation of the oil, the total diene material present was reduced and the concentration of the conjugated diene material was increased to as much as 16% of the total diene material present. The data are summarized in Table 1.

The rate of loss of unsaturated fatty acids is of major importance nutritionally if it is assumed that the material measured represents the "essential" unsaturated linoleic and linolenic acids of Burr, *et al.* (16). It is realized, however, that the spectroscopically active di- and triethenoid material measured in these studies may not be the same as the biologically active forms.

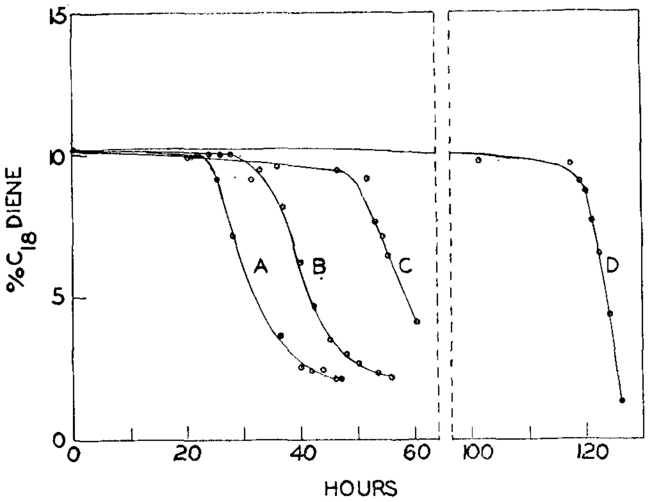

FIG. 6. Rate of loss of C_{18} diene acids (determined as esters) during fat autoxidation.

A. Shortening A
B. Shortening B
C. Shortening B + ascorbyl palmitate
D. Shortening B + gallic acid

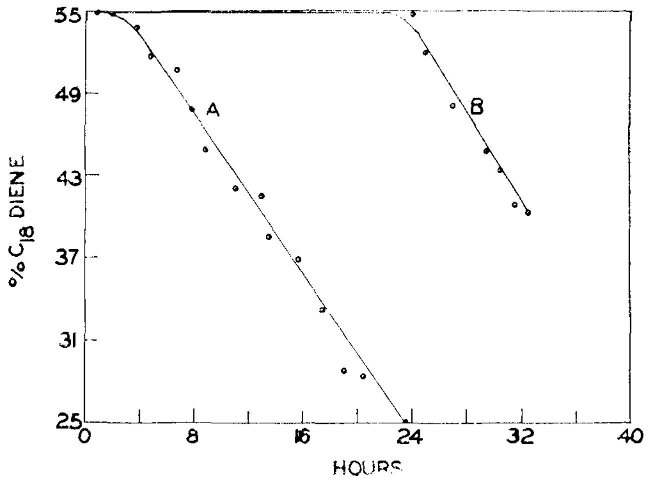

FIG. 7. Rate of loss of C_{18} diene acids (determined as esters) during fat autoxidation.

A. Cottonseed oil
B. Cottonseed oil + gallic acid

When the concentration of diene acid (calculated as methyl esters) was plotted as a function of time, curves typical of an autocatalytic reaction were obtained (Figures 6 and 7). Rapid loss of diene acids occurred at the end of the induction period. The loss of total diene material at the end of the induction period was 10% in the case of hydrogenated shortenings and 2% in the case of the cottonseed oil. The latter, however, had a higher initial concentration of diene acids. Thus, a sample undergoing autoxidation may become rancid but retain the major portion of its diene acids.

The concentration of triene material increased with time until at maximum peroxide value it was about five to six times its original concentration. This increase has been attributed to the occurrence of some dehydrogenation of the substrate. The enhanced absorption of ultra-violet light in the region of 270 mμ may also be due to cyclized dimers or hydroperoxides spectroscopically active in this region.

The resonance of carbonyl groups present in the decomposition products of fat deterioration also absorbs in this region of the ultra-violet, and this effect more probably explains the rise in general absorption (17).

When the amounts of linoleic acid (as methyl esters) of the various samples of a given system were plotted against the peroxide values (Figures 8 and 9), it was observed that the fore part of the curves approached a straight line. This same phenomenon occurred when the amounts of linoleic acid were plotted against iodine values (Figures 10 and 11).

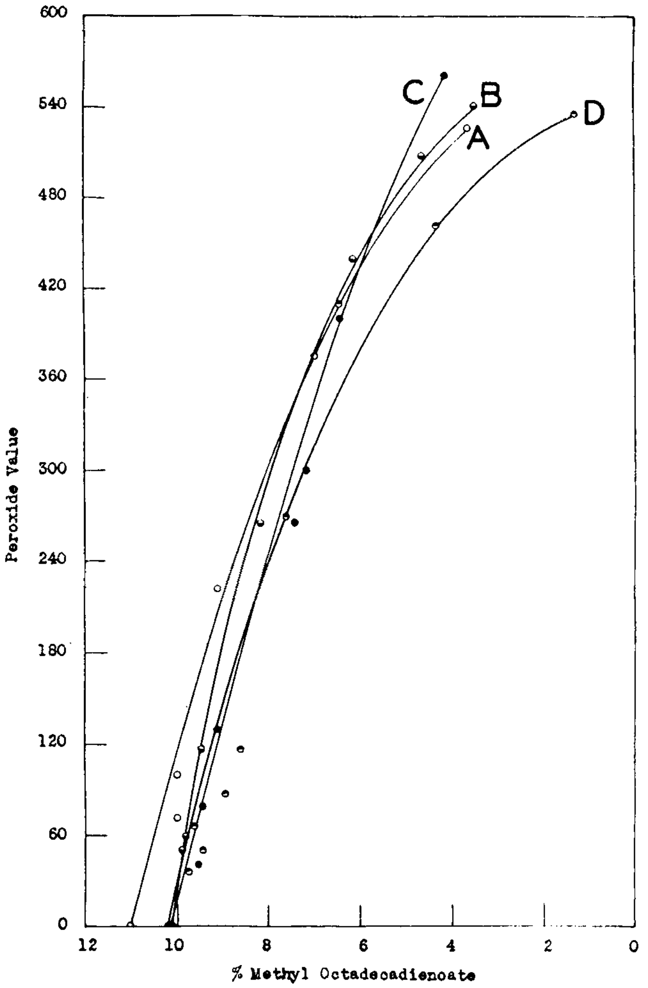

FIG. 8. The change in concentration of diene acids as a function of peroxide value.

A. Shortening A
B. Shortening B
C. Shortening B + ascorbyl palmitate
D. Shortening B + gallic acid

From these observations it is apparent that during the early stages of the oxidation, up to a peroxide value of 300 to 400, linoleic acid was the only unsaturated component being destroyed. In no case was there an appreciable amount of linolenic acid present. This conclusion was given support when the per cent of oleic acid in the various samples was calculated.

Some interesting phenomena were observed when the concentration of oleic acid in the various samples of a given system were plotted against the lengths of time the samples had been aerated (Figure 12). With the hydrogenated vegetable oil shortenings, where the oleic acid content was high and the linoleic acid content low, it may be observed that the amount of oleic acid remained constant for a considerable length of time before beginning to disappear. In fact, the curves have the appearance of typical induction period curves. The first appreciable loss of oleic acid occurred only after the peroxide value of a sample had risen to 300 to 400. Once the destruction of oleic acid had begun, it proceeded at a rapid rate.

What appeared to be anomalous results were obtained when the concentration of oleic acid in the samples of refined cottonseed oil were calculated and plotted against hours of aeration (Figure 13). The determined iodine values and linoleic and linolenic

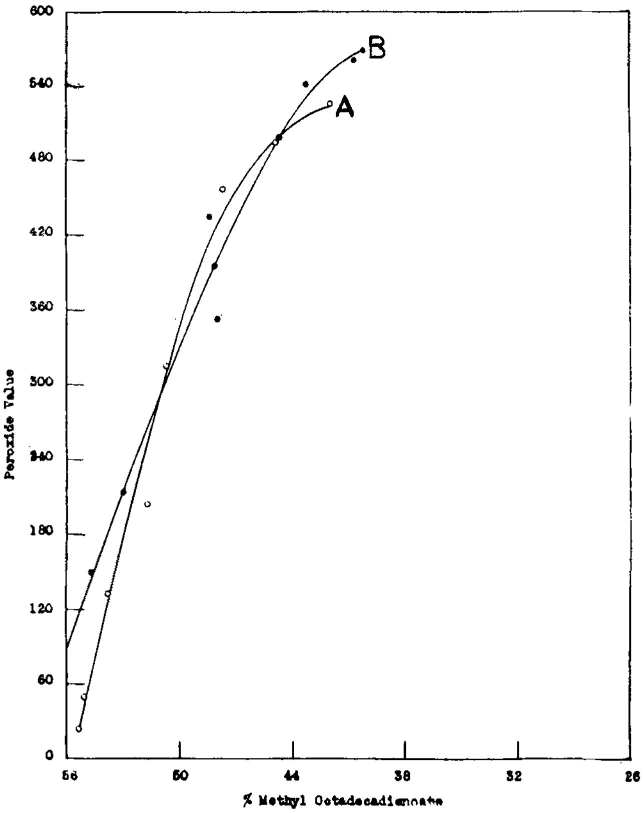

FIG. 9. The change in concentration of diene acids as a function of peroxide value.

A. Cottonseed oil
B. Cottonseed oil + gallic acid

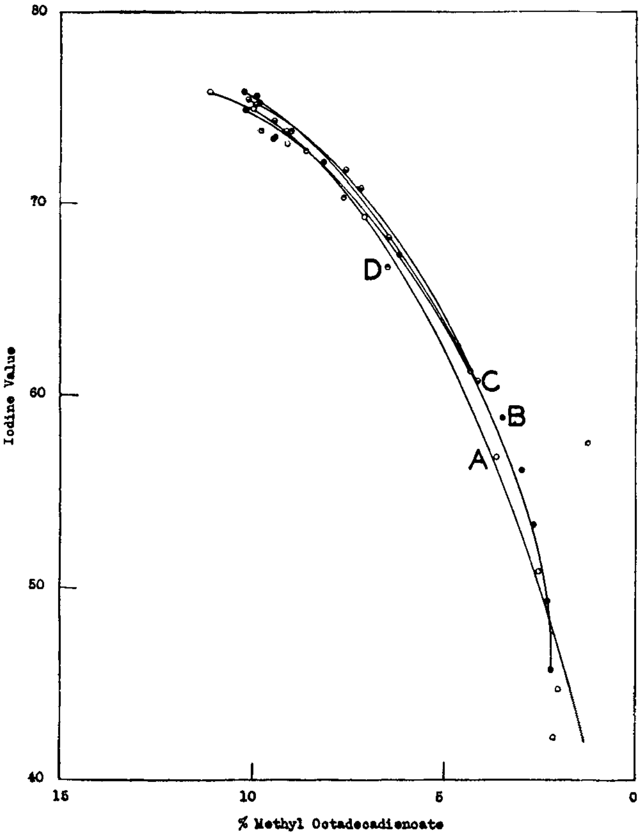

FIG. 10. The change in concentration of diene acids as a function of iodine value.

A. Shortening A
B. Shortening B
C. Shortening B + ascorbyl palmitate
D. Shortening B + gallic acid

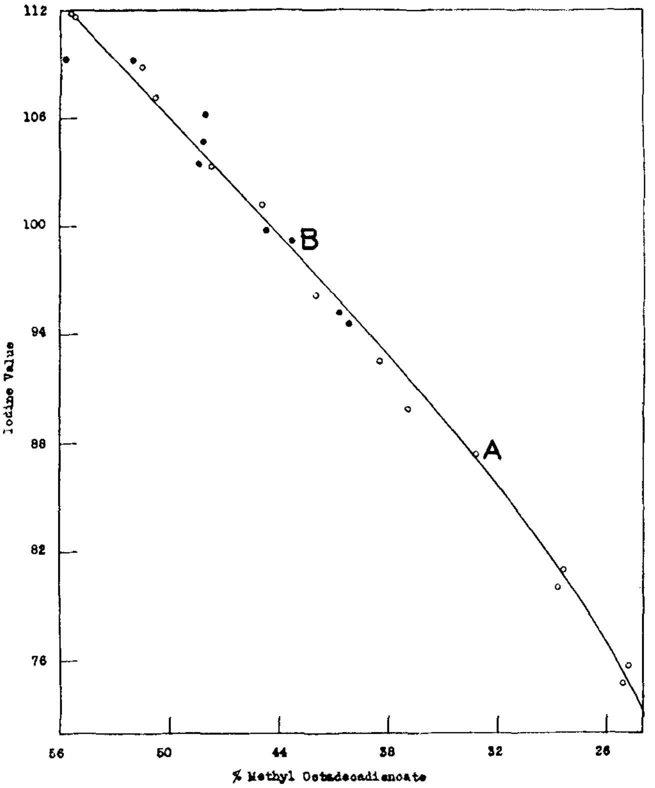

FIG. 11. The change in concentration of diene acids as a function of iodine value.

A. Cottonseed oil
B. Cottonseed oil + gallic acid

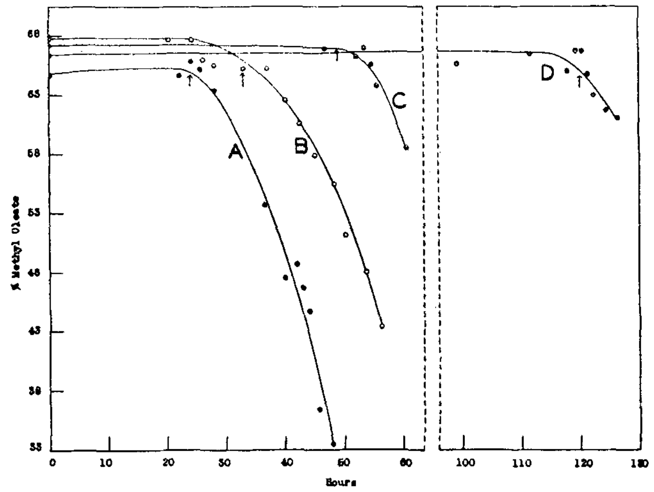

FIG. 12. Decrease in oleic acid (calculated as methyl oleate) during autoxidation of hydrogenated shortenings.

A. Shortening A
B. Shortening B
C. Shortening B + ascorbyl palmitate
D. Shortening B + gallic acid
↑ End of induction period (P.V. = 120)

acid contents were such that the proportions of oleic acid appeared to increase as the time of oxidation was prolonged. This seemed to indicate that in the oxidation of linoleic acid there was formed some unsaturated compound or compounds which absorbed iodine but which were not measured spectrophotometrically as linoleic acid. The fact that such a phenomenon occurred in the case of the oil and not with the hydrogenated vegetable oil shortenings may be attributed to the greatly different ratios of linoleic acid to oleic acid in the fat substrates.

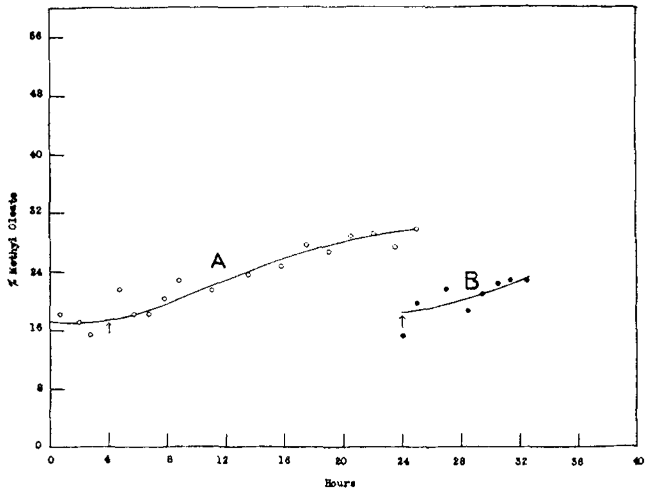

FIG. 13. Change in oleic acid (calculated as methyl oleate) during autoxidation of cottonseed oil.

A. Cottonseed oil
B. Cottonseed oil + gallic acid
↑ End of induction period

It is somewhat surprising that the amount of oleic acid should increase. This may be explained on the assumption that the dehydrogenation of the substrate proceeds at a greater rate than the decomposition of the oleic acid.

Summary

A general pattern of chemical changes has been observed to accompany the oxidative destruction of a fat during the accelerated development of rancidity in the presence of oxygen at 110° C. During a variable period of time (the "induction period") no detectable changes occur. However, at the time when peroxide formation increases appreciably, several chemical changes appear simultaneously: linoleic acid decreases, the total unsaturation (as measured by the iodine value) decreases and the mean length of the carbon chain of the acids decreases. The formation of conjugated unsaturation in an autoxidizing edible fat or oil seems probable. It has been demonstrated that a fat may become quite rancid and still retain at least 90% of its original octadecadienoic, "linoleic" acid. These chemical changes have been observed consistently irrespective of the fat or oil under study, the length of its induction period, or the presence of added antioxidant or synergist.

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