834

FAT RANCIDITY

Recent Studies on the Mechanism of Fat Oxidation in Its Relation to Rancidity

STEWARD G. MORRIS
Eastern Regional Research Laboratory, Philadelphia 18, Pa.

The autoxidation mechanism, as now generally accepted, is a chain reaction involving radicals formed at the α-methylene groups adjacent to the double bonds. The radicals consume oxygen and then react with another olefin to give hydroperoxide and another free radical. Each free radical is a resonance hybrid compound of three equivalent structures. The displacement of double bonds occurring in oxidation can be accounted for by this free radical mechanism. By the use of new or improved techniques, evidence has been attained to support the fundamental concepts. Chromatography and countercurrent distribution methods have been applied to the isolation and identification of oxidation products. Polarographic methods have been developed for the determination of hydroperoxides, and in the presence of oxidation decomposition products may be more specific than the chemical method. Ultraviolet spectrophotometric analyses have aided in the identification of oxidation products and in revealing double bond shifts which take place in polyunsaturated fatty acids. Infrared spectrophotometry was useful in determining changes in geometrical configuration of fatty materials and in detecting functional groups. Data obtained by these techniques by numerous workers are discussed in relation to the mechanism of oxidation of mono-, di-, and triethenoic fatty acids.

American and English literature pertaining to the autoxidation of fats is the chief concern of this review. The mechanism of oxidation has been discussed (*43*, *46*, *52*, *60*). Consequently this paper is limited, in so far as practicable, to review of the work of the past 5 or 6 years.

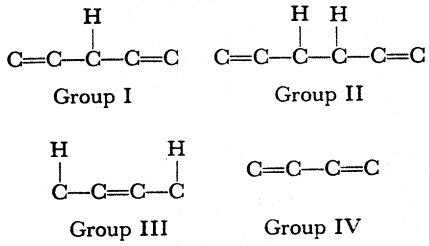
The mechanism of oxidation of fats is extremely complex, especially when the fat is highly unsaturated and consists of many different acids and esters. The tendency has been to study purer compounds and known mixtures, so that the products of oxidation would be less numerous and more easily isolated and identified, and the data obtained would be more subject to interpretation.

The use of purer compounds and known mixtures has become possible with the development of new or improved techniques in low-temperature crystallizations, chromatography, countercurrent distribution, polarography, and spectrophotometry in the ultraviolet and infrared ranges. Most of the work here reviewed has been dependent on one or more of these procedures.

The theory of peroxide formation was proposed as early as 1897 (*3*, *18*). A peroxide formed by autoxidation was not isolated, however, until 1928, when Stephens (*55*) prepared cyclohexene peroxide, which he believed to be a cyclic peroxide across the double bond. Criegee *et al.* (*15*), however, showed that cyclohexene hydroperoxide still contained a double bond, and this was confirmed by Farmer and Sundralingam (*23*) in 1942. Then followed several papers by Farmer and coworkers (*20*, *22*, *24*) which are chiefly responsible for the formulation of the present accepted theory of autoxidation of fats.

For many years, the reactions at ethylenic bonds of simple olefins were considered to be additive reactions with polar reagents. When the molecule was unsymmetrical about the double bond, the direction of addition of a polar reagent such as HX to the double bond became precisely determined in terms of the well-known Markovnikov rule. In the study of autoxidation, however, Farmer (*19*) pointed out that there had been a steady accumulation of experimental results which did not fit into the polar scheme but which could be interpreted as resulting from the occurrence of a polar type of reaction in which the participants were short-lived neutral entities (molecules and atoms) displaying free-radical characteristics. The formation of free radicals from fat molecules is dependent on hydrogen lability. The latter is determined largely by the type of

unsaturation pattern and also by the presence or absence of substitution on the ethylenic carbon atoms. Farmer arranged into groups, according to hydrogen lability, the following four types of unsaturated systems:

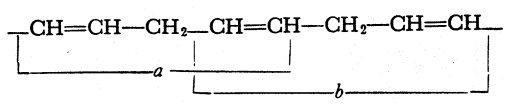


Owing to an α-methylene group flanked by adjacent double bonds, Group I has the highest degree of hydrogen lability. Group II shows less lability, because the influence of the two double bonds is divided between two methylene groups. Group III shows still smaller hydrogen lability, and Group IV, being a conjugated olefin, has lost most of its hydrogen lability.

The generally accepted theory of autoxidation of fats, especially in the initial stages of oxidation, is based on the pentadiene system (Group I). A free radical first forms on the α-methylene group, which sets up resonance isomers with either of the adjacent carbon-to-carbon unsaturated groups. The free radical absorbs an oxygen molecule, which then accepts hydrogen to form a hydroperoxide. The hydrogen usually comes from another fatty acid or ester to form another free radical, and thus the chain reaction is maintained. The isomerization or shifting of double bonds that takes place in some autoxidations is also supported by this theory.

In the oxidation of monoenoic long-chain acids having less active methylene groups, as in Group III, Farmer (*21*), Bolland and Gee (*9*), and simultaneously Gunstone and Hilditch (*28*) came to the conclusion that the point of oxidative attack was at the double bond instead of the α-methylene group. Their conclusions were based on thermodynamic data and on the fact that initiation of oxidation of methyl oleate was greatly accelerated by the addition of small amounts of methyl linoleate. They agreed, however, that only a slight amount of such oxidation was required to form sufficient free radicals to permit the usual α-methylenic free radical chain reaction to proceed.

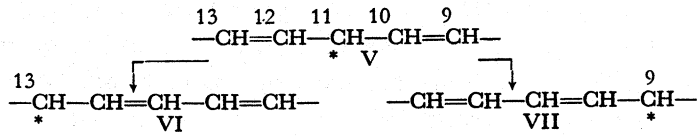
In discussing the mechanism of oxidation as it applies to the various types of unsaturated fatty acids, it seems appropriate to consider first the dienoic acid, linoleic, because it contains the active pentadiene —CH=CH—CH_2—CH=CH— group. This unsaturated system might be considered as a basic functional unit in the addition of oxygen or autoxidation. Hilditch (*30*) suggests that this system is also intimately connected with the addition of hydrogen in the selective hydrogenation of fats. Furthermore, linolenic acid with its octatriene system could be thought of as having two pentadiene systems, depending upon whether the *a* or *b* portion is being considered.



Evidence supporting such a conception is presented later.

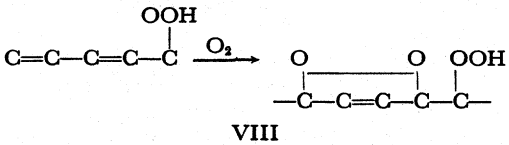
Dienoic Acids

In a discussion of the theory of oxidation of the dienoic acids, linoleic acid is the most important, and it has received the most attention in experimental work. Its unsaturated system, according to Farmer's theory, would permit the formation of three possible free radicals with their corresponding hydroperoxides (V, VI, VII):



Thus, a monohydroperoxide might be formed on carbons 11, 13, or 9. If formed at 11, the compound would be nonconjugated; if at 9 or 13, the hydroperoxide would be conjugated. Bergström (*5*) obtained data indicating that hydroperoxides were formed at carbons 9 and 13 but was unable to show that any hydroperoxide was formed at carbon 11.

Lundberg *et al.* (*44*) prepared hydroperoxides of methyl linoleate and concentrated them at low temperatures to obtain practically pure monohydroperoxides (peroxide value of 6268 meq. per kg. or 1.02 moles per mole of ester). The amount of conjugated peroxides was considerably less than the total peroxides, indicating that there must be some unconjugated peroxides present—possibly the 11-position hydroperoxide. They also found that the hydroperoxide concentrates oxidized readily (not when diluted with unoxidized methyl linoleate) to form diperoxides of the possible structure



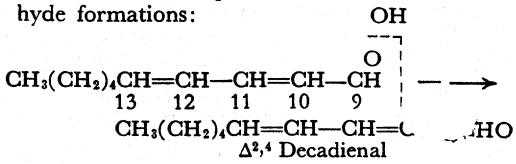
This cyclic diperoxide structure had been proposed earlier by Dugan and coworkers (*16*). They postulated that if conjugated hydroperoxides should absorb oxygen in the 1–4 positions, decreased absorption at 2310 to 2320 A. would result. This would account for the presence of two different bands, on a chromatographic adsorption column, having equally high peroxide values, but the more polar adsorbate at the top of the column showed much less absorption at 2310–20 A.

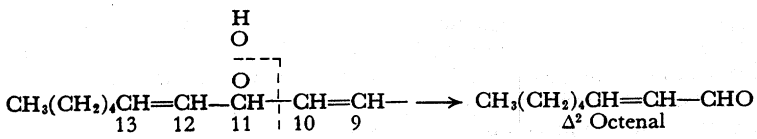
Cannon *et al.* (*12*), working with concentrates of hydroperoxides prepared by countercurrent extraction, found the hydroperoxides to be 90% conjugated. Thus, the 11-position hydroperoxide formed only in small amounts, if at all. Higher oxidation levels showed that the diene conjugation dropped to only 17 to 22%. After mild hydrogenation of this fraction, approximately one hydroxyl group was present per mole of ester. If the oxygen ring structure is stable under the conditions of peroxide determination and hydrogenation, these facts are in agreement with VI, VII, and VIII.

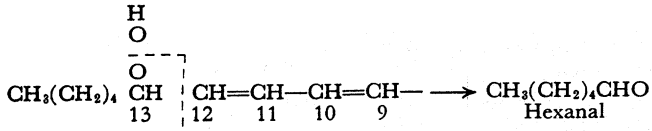
A study of the decomposition products of autoxidation has materially added to our knowledge of the mechanism of oxidation. It has long been known that aldehydes are produced during oxidation of fatty materials. Swift and associates (*62*) identified one saturated and two unsaturated aldehydes as oxidation products from decomposed hydroperoxides of cottonseed oil, linoleic acid being the principal fatty acid constituent as well as the most readily oxidized component of the oil.

Autoxidized cottonseed oil was steam-distilled to decompose the hydroperoxides and to separate the volatile aldehydes. The semicarbazones of hexanal, $\Delta^{2,4}$ decadienal, and Δ^{2} octenal were crystallized from the steam distillate. The structures of the aldehydes were determined on the basis of the absorption spectrum of their semicarbazones, and the chain lengths were determined by reduction to and identification of the corresponding *n*-aliphatic 2,4-dinitrophenylhydrazones.

Isolation of the three aldehydes was offered as evidence for the possible presence of 9, 11, and 13 hydroperoxidolinoleates in the oxidation mixture. The following reactions, which cannot be represented stoichiometrically, offer an explanation of the aldehyde formations:







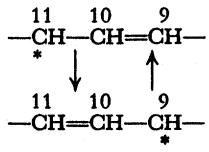
In view of the fact that $\Delta^{2,4}$ decadienal exhibits absorption at 273 to 274 mμ, Swift suggests that this compound may account for the development of absorption bands in the region ca. 275 mμ, which has been noted in oxidizing fat systems by several investigators.

Brekke and MacKinney (*10*) prepared aldehyde derivatives from steam distillates of rancid corn and avocado oils, in which they confirmed the above-mentioned findings of Swift *et al.* (*62*).

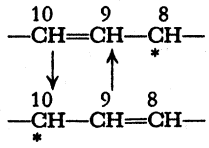
Monoenoic Acids

Study of the oxidation of the relatively simple monoenoic fatty acids, such as oleic, has given rise to more apparently conflicting data than has the corresponding study of the dienoic acids.

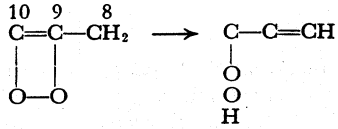
Farmer and Sutton (*24*) isolated from oxidized methyl oleate a monohydroperoxide that retained its olefinic unsaturation. Evidence indicated that there were two isomeric monohydroperoxides, which had formed at carbons 8 and 11. Later Farmer, Koch, and Sutton (*22*) proposed a free radical mechanism for mono-olefinic acid oxidation involving two three-carbon systems in resonance. The oleic acid group $\overset{11}{-CH_2}-\overset{10}{CH}=\overset{9}{CH}-\overset{8}{CH_2}-$, for example, would have the free radicals:



and



Hence, according to this postulation, hydroperoxides could be formed at carbons 8, 9, 10, and 11. As has been pointed out (*20*), later Farmer changed his opinion regarding the oxidation of monounsaturated systems. Gunstone and Hilditch (*28*) disagreed with this theory also, and suggested that oxygen molecules are primarily attached to an enoid bond, and not to an adjacent methylene group, hydroperoxide finally being formed with the formation of a new ethenoid bond.

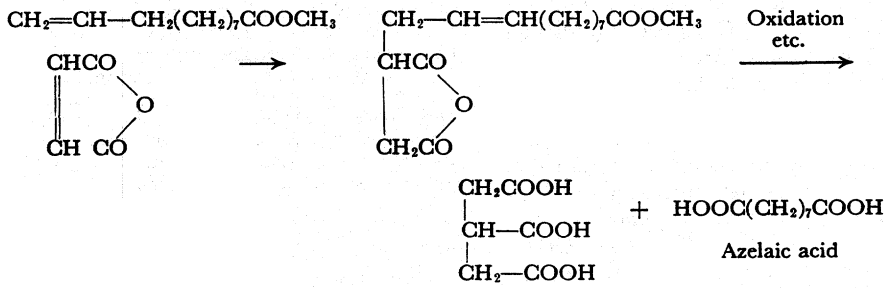


This theory would provide for formation of hydroperoxides only on carbons 9 and 10.

In answering the question whether oxygen adds to the double bond of oleic acid to form hydroperoxides only at carbons 9 and 10 or whether oxygen attaches to the methylene groups adjacent to the double bond in a resonance system to form four possible monohydroperoxides at carbons 8, 9, 10, and 11, the position of all the hydroperoxides formed by autoxidation must be determined.

In attempting to determine the structure of hydroperoxides of fatty acids, several workers have made use of the close analogy between the behavior of oxygen and maleic anhydride toward unsaturated compounds. The similarity of their behavior has been well substantiated (*8, 20, 32*).

Ross *et al.* (*49*) prepared a maleic anhydride addition product with undecylenic and oleic esters.



Oxidation of the adduct caused rupture at the double bond with the production of tricarballylic and azelaic acids, demonstrating the structure of the adduct. Maleic anhydride and oleic ester yielded adducts by addition at the double bond at either carbon—namely, carbons 9 and 10. It seemed obvious at the time that the point of addition was at the double bond, with subsequent shifting of the bond to the β, γ position, the point of addition being α. From these data one could readily infer that autoxidation of oleic acid took place at the double bond, with simultaneous shifting of the original double bond. These data, however, do not preclude the possibility that oxidation and addition of maleic anhydride occur instead on the methylenic group adjacent to the double bond. The theory of free radical chain mechanism (*22*) assumes that the points of addition could be on carbons 8, 9, 10, and 11 for oleic acid.

Bickford (*7*) and his coworkers treated methyl oleate with maleic anhydride, and from the adduct mixture they were able to isolate and identify the structure of four isomeric maleic anhydride–methyl oleate adducts. The addition took place on carbons 8, 9, 10, and 11 of the oleic acid, and the isomers were produced in nearly equal amounts.

In view of the similarity between the reactions of maleic anhydride and those of oxygen with unsaturated compounds, this work suggests that the free radical mechanism of autoxidation is correct and that four isomeric hydroperoxides are formed during the autoxidation of methyl oleate.

Ross, Gebhart, and Gerecht (*50*) examined the autoxidation of methyl oleate with oxygen in the presence of ultraviolet light at 35° C. They found evidence that all four of the monohydroperoxides suggested by the chain mechanism were present. The hydroperoxide groups were located on carbons 8, 9, 10, and 11 of oleic acid, and the yields, in diminishing order, were carbon 10, 11, 8, and 9.

It was thought that by labeling the carbons at the double bond and also those carbons adjacent to the double bond by substituting deuterium for hydrogen, information might be gained concerning the induction period, peroxidation, polymerization, and fragmentation of the molecule giving rise to rancidity. Accordingly, Khan *et al.* (*36*) and Max and Deatherage (*45*) did some excellent synthetic work in preparing methyl 9,10-dideutero-oleate and 8,8,11,11-tetradeutero-*cis*-9-octadecene. The results of autoxidation, however, were not conclusive, and re-emphasized the complexity of the autoxidation phenomenon. In fact, the inconclusive results obtained when carbons 9 and 10 were loaded with deuterium are another indication that carbons 8 and 11 are also involved in autoxidation.

By the analysis of oxidized methyl oleate by countercurrent distribution

methods, Fugger *et al.* (*26*) obtained data to support the view that monohydroperoxides are the first stable products in the reaction with gaseous oxygen and that the ethylenic bond is not attacked until a subsequent oxidation.

Relatively little is known concerning the secondary stages of autoxidation. It is often assumed that hydroperoxides react at the olefinic linkages to produce epoxides. Swift (*61*) used an ingenious method to study the effect of methylhydroperoxido-oleate as an oxidizing agent. Methylhydroperoxido-oleate and oleic acid were mixed and stored at 90° C. in an atmosphere of nitrogen for 3 days. The acid was separated again from the esters by alkaline extractions. It was found that the oleic acid was oxidized at the double bond by the methyl hydroperoxido-oleate to form 9,10-epoxyoleic acid and the low melting form of 9,10-dihydroxystearic acid. None of the high melting form of 9,10-dihydroxystearic acid was isolated. In most of the catalytic oxidations reported by Swern *et al.* (*59*) and Skellon (*53*), the yield of high-melting form predominated.

It is now generally accepted that autoxidation of unsaturated fatty compounds is initiated and propagated through an active methylene group adjacent to a double bond. This is especially true when the methylene group is adjacent to and separates two unsaturated centers. Doubt has been expressed by several workers, who question whether the CH_2 group adjacent to a single double bond (as in monounsaturate long-chain fatty acids) has sufficiently labile hydrogen to initiate oxidation (*9*, *21*, *28*). In fact, Hilditch (*31*) has come to the conclusion that highly purified methyl oleate that contains no trace of linoleate or other polyunsaturated fatty compounds does not autoxidize at 50° C. or below (although at higher temperatures direct union between oxygen and methyl oleate proceeds rapidly) (*2*, *27*). He suggests that "...at ordinary temperatures, initiation of autoxidation is a function solely of long-chain unsaturated compounds which contain a pentadiene system, —CH=CH—CH_2—CH=CH—, with a central reactive methylene group..." The course of oxidation of oleate groups is postulated as follows:

$$RH \longrightarrow R\text{—};\ R\text{—} + O_2 \longrightarrow ROO\text{—};$$
$$\text{—}CH_2\text{—}CH{=}CH\text{—}CH_2\text{—} + ROO\text{—} \longrightarrow \text{—}CH_2CH{:}CH\text{—}\underset{\underset{OOH}{|}}{CH}\text{—} + R\text{—}$$

where RH is initially methyl linoleate, but may afterward also be methyl oleate.

Reasons for this conclusion are the marked resistance of highly purified methyl oleate to autoxidation at 20° C. in diffused daylight and the observation (*28*) of the marked effect of 1% or less of added linoleate in promoting oxidation of methyl oleate. Hilditch believes that much of the methyl oleate used in earlier experiments contained some linoleates. He states, "....the explanation now offered not only clarifies somewhat divergent observations, but also points to a major difference in behavior to oxygen (as might be expected) between the isolated monoethenoid system, —CH_2—CH=CH—CH_2, and the pentadiene grouping, —CH=CH—CH_2—CH=CH—, with its 'reactive' central methylene group."

Trienoic Acid

The mechanism of oxidation of the linolenates has not been studied so extensively as that of the oleates and linoleates. It has been assumed that the oxidation of the linolenates followed the same pattern as that established for the mono-olefins and nonconjugated diolefins.

Most of the recent studies of the linolenates has consisted of fractionation of oxidized materials leading to isolation and identification of the products of oxidation. Development of the countercurrent extraction procedure has greatly facilitated these studies, in that it permits examination of all the components of the oxidation mixture and it is also an extremely mild method of fractionation.

Fugger and coworkers (*25*) fractionated autoxidized methyl linolenate by countercurrent distribution techniques using 80% aqueous ethyl alcohol and hexane as solvents. Their results showed that the course of oxidation of methyl linolenate differed significantly from that reported for methyl oleate and methyl linoleate. They concluded from the analytical studies that:

1. Monomeric monohydroperoxide, if present, exists in very small quantity.
2. Dimers are formed during the oxidation process or immediately thereafter.
3. Less than half the linolenate actually oxidized is converted to a conjugated form, and more than half the double bonds originally present are destroyed, probably through polymerization initiated by oxidative attack on the ethylenic bonds.

Many of the recent studies of the linolenates have been directed toward determining the cause of "reversion" flavors in soybean oil. Circumstantial evidence has long pointed to linolenic acid as the precursor of the product or products causing the reversion. Circumstantial evidence against linolenic acid was extended by Dutton *et al.* (*17*), who interesterified methyl linolenate with the glycerides of cottonseed oil. Organoleptic tests indicated that the treated cottonseed oil developed reversion flavors. Thus it was concluded that linolenic acid was the unstable precursor of "fishy-painty-grassy-melony" flavors in soybean oil.

A study of the flavor materials in reverted soybean oil was made by Schepartz and Daubert (*51*) and Stapf and Daubert (*54*). The flavor mater were obtained in steam distillates fr reverted soybean oil. They identified acetaldehyde and $\Delta^{2,4}$ decadienal as possible contributors to the reversion flavor.

As noted before, $\Delta^{2,4}$ decadienal had already been shown to be derived from the oxidation of linoleic acid (*62*). This is an indication that acids other than linolenic might be responsible for reversion flavors.

Kawahara and Dutton (*35*) collected the volatile odor principles of reverted soybean oil and fractionated them into rancid and reversion components by adsorption on silica gel. These principles apparently contained aldehyde groups, for they could no longer be detected organoleptically after the addition of aldehyde reagents. The aldehydes were identified as acetaldehyde, propionaldehyde α-pentenal, and hexanal. The formation of all these aldehydes except hexanal can be explained as cleavage products of isomeric hydroperoxides of linolenic acid. Hexanal had previously been isolated from oxidized cottonseed oil (*62*).

n-Hexanal has been identified as a constituent of the painty fraction (*35*) and 2-heptenal as a reversion compound of (*5* soybean oil. Both these aldehydes wei obtained by Chang and Kummerow (*14*) as the volatile decomposition products from oxidative polymers and hydrogenated oxidative polymers of ethyl linoleate. The polymers, consisting of dimers and trimers, were prepared by autoxidation of ethyl linoleate at 30° C. for 250 hours.

The polymers in pure form had a bitter, painty, and rancid odor and flavor; when added to fresh shortening in 1000 p.p.m., they made the mixture definitely rancid. On the basis of these facts, these investigators suggest that linoleic acid could be one of the precursors of reversion flavors, in spite of the fact that oils which contain no linolenic or other more highly unsaturated acids do not revert easily. It is known that the behavior of mixtures of esters does not always parallel the behavior of pure esters. Therefore, it is possible that the linolenate in soybean oil accelerates the autoxidation of linoleate, with the formation of polymers, which in turn decompose and yield these aldehydes.

Rates of Oxidation

Accurate comparisons of oxida. rates for various unsaturated fatty acids

Table I. Alkali-Isomerization-Spectrophotometric Analysis of Methyl Linoleate and Methyl Linolenate Autoxidized at 0° C.

Peroxide Value, Meq./Kg.	Oxidized, %	"Linoleic," %	"Linolenic," %	"Arachidonic," %	Conjugated Diene, %	Conjugated Triene, %
			Methyl Linoleate			
0	0	99.86	0	0	0.084	0.009
158	2.6	98.45	0	0	1.5	0
965	16.1	86.68	0.108	0.022	9.39	0
1470	24.5	78.21	0.97	0.032	14.70	0
			Methyl Linolenate			
0	0	0	100.2	0	0.17	0
168	2.8	0	97.68	1.29	1.31	0
800	17.0	0	88.39	6.65	5.26	0
2300	38.3	0	66.89	17.83	15.71	0.711

and esters cannot be made from the literature, for much depends upon the temperature and the manner of relating time with peroxide data. A number of investigators (*27*, *34*, *56*) have shown, however, that the linolenate oxidizes approximately twice as fast as the linoleates. This may be correlated with the fact that linolenate has twice as many active methylene groups as has the linoleate. In oleate, where there are no methylene groups between unsaturated carbons, the oxidation is reported as ranging from one twelfth to one fiftieth that of linoleate. When the double bonds in fatty acids are separated by more than one methylene group, the reaction rate for each double bond approaches that of monounsaturated acids. Allen *et al.* (*1*) also found that when the active methylene group is missing, as in conjugated $\Delta^{10:12}$ linoleic acid, the oxidation rate is one third that of the nonconjugated $\Delta^{9:12}$ linoleic acid. Furthermore, the $\Delta^{10:12}$ linoleic acid absorbed 1 mole of oxygen per mole of ester before any appreciable formation of peroxides took place.

Hilditch (*30*) has pointed out the similarity of the rates of selective hydrogenation and of autoxidation. Bailey and Fisher (*4*) in studying selective hydrogenation found that methyl linolenate hydrogenated twice as fast as the linoleate and forty times that of the oleate.

Ultraviolet Spectrophotometry

Spectrophotometry in the ultraviolet range has become extremely valuable in fat analysis, especially within recent years as more accurate constants for various fatty substances have been established through the use of more highly purified reference compounds (*11*, *29*).

The effect of autoxidation on the absorption characteristics of fatty materials was pointed out by Swain and Brice (*57*). They showed that small amounts of conjugated triene were formed during alkali-isomerization of autoxidized dienoic acid and similarly tetraene from autoxidized trienoic acid. Holman (*33*) also reported that the monohydroperoxides formed from linoleic acid during autoxidation are completely conjugated, at least as far as 30% oxidation, at 0° C. Allen (*1*) stated that oxygen is as effective as alkali in isomerizing the CH=CH—CH_2—CH=CH system to the conjugated system, although the rate is slower. Privett and Lundberg (*47*) studied the effect of autoxidation on the ultraviolet absorption of methyl linoleate and linolenate. Table I shows some of the noted examples reported in their tables. Methyl linoleate, having zero peroxide value, had less than 0.1% conjugated diene, but when oxidized to 1470 meq. per kg., it contained 14% conjugated diene. For methyl linolenate, there was a continual development of tetraene as well as conjugated diene during the autoxidation.

Infrared Spectrophotometry

Development of the infrared spectrophotometric technique has provided a method for the detection and quantitative determination of trans isomers in the presence of large amounts of cis isomers. Knight *et al.* (*37*) followed the autoxidation reaction of methyl oleate by means of infrared spectrophotometry. They found that all, or nearly all, of the peroxides produced during the oxidation at 35° C. in the presence of ultraviolet up to 300 hours were trans peroxides. Thus the original cis configuration of the methyl oleate shifted to the trans isomer as the hydroperoxide oleate was formed. Cannon (*12*) found that the autoxidation of methyl linoleate at room temperature to the low level of 0.11 mole of oxygen per mole of ester yielded only hydroperoxides that were 90% cis-trans conjugated isomers. Privett and coworkers (*48*) reported similar results for the oxidation of methyl linoleate at 0° C. in the dark. However, when the oxidation took place at 24° C. in the dark to an oxidation level of 325 meq. per kg., the infrared analysis indicated that appreciable amounts of conjugated trans-trans hydroperoxides were present in addition to those of the cis-trans type. They suggest that possibly the conjugated cis-trans isomers were formed originally but were labile at the higher temperature in the presence of catalyst—e.g., peroxides—and were transformed to the thermodynamically more stable conjugated trans-trans isomer.

Polarography

Lewis and coworkers (*39*, *40*) outlined a polarographic procedure that permitted the study of water-insoluble peroxides such as those occurring in fats, ethers, and hydrocarbons. Willits *et al.* (*64*) extended the application of the polarograph in the study of fat autoxidation products by determining the polarographic waves of 41 pure oxygen-containing compounds. The compounds contained functional groups which are known or suspected to occur in autoxidized fats.

When the chemical method (*63*) and polarographic method were compared for α-pinene hydroperoxide, *p*-menthane hydroperoxide, pinane hydroperoxide, and methyl oleate hydroperoxide of 96.5% purity, the peroxide values were practically identical. When the two methods were compared by determining the peroxide values in fat oxidation mixtures, the agreement was not so good. This was to be expected, except that the differences were not consistent. In the oxidation of methyl oleate (*65*) at 80° C. to a peak peroxide value of 2002 meq. per kg. (polarographic value) the relative per cent differences for the two methods, for seven samples taken at intervals, ranged practically uniformly between +6 and −6. This indicated that the two methods were measuring the same reducing compounds but that the relative agreement was about 12%. In another experiment by Swern *et al.* (*58*), in which methyl oleate was autoxidized at temperatures from 35° to 120° in the presence of ultraviolet radiations but only to a maximum oxidation level of 87 meq. per kg., the polarographic peroxide values were consistently lower. In five determinations, the greatest difference amounted to 24%. In the autoxidation of methyl linoleate (*66*) to a peak chemical peroxide value of 4300 meq. per kg., the time-peroxide curves for the two methods agreed fairly well, except that the polarographic values were consistently lower by approximately 14%. When the values had dropped from the peak values to 3000 meq. per kg. with continued oxidation, the polarographic peroxide value was then 20.7% higher than the chemical peroxide values.

It appears to the writer that the polarographic method for determining hydro-

peroxides in autoxidation of fats may be more accurate than is the chemical method with which it is here compared. An excellent beginning has been made in the development of the polarographic method for autoxidation studies of fats; its progress is largely dependent upon the preparation and isolation of pure compounds of autoxidation that can be used as reference compounds.

Rancidity

The question arises concerning the mechanism of autoxidation and its relationship to the mechanism of the development of rancidity. It is true that most of the studies of autoxidation are carried out on fats that are oxidized far beyond the rancid stage. This is necessary in order to obtain oxidation products in sufficient amounts for chemical study. The sense of taste is so much more sensitive than chemical reactions that the offending flavors may be detected when only a few parts of the products per million are present. Definite rancidity may occur when 0.1% of fat undergoes chemical change (*41*).

It seems reasonable to assume, however, that the compounds responsible for rancid odors and flavors are formed in larger amounts as oxidation proceeds. This is borne out organoleptically, since the rancid odors and flavors become stronger with continued autoxidation. Thus, it would appear that the studies previously made are directly applicable to the rancidity problem.

All fats, in the presence of oxygen, are subject to oxidative rancidity and some are also subject to flavor reversion. Oxidative rancidity is due to a breakdown of the primary oxidation products of fats to form a variety of shorter chain compounds. On the other hand, flavor reversion was defined by Lips (*41*) as a flavor deterioration caused by less oxygen than is required to produce true oxidative rancidity. The importance of linolenic acid in the development of reversion flavors has been discussed in this paper.

Development of rancidity of fats in foods often involves an aqueous fat system. Comparatively little work has been done in such systems, and it is often difficult to draw any general conclusions, as moisture may influence the solubility of accelerators or inhibitors. Lea (*38*) in reviewing the literature found that the fat in most powdered foods, crackers, and other cereal foods becomes more rancid as the moisture content is lowered to extremely low values. In contrast, moisture was shown to have an accelerating effect on the oxidation of lard. Chang and Watts (*13*) observed that the effect of sodium chloride on fat oxidation depended greatly on the amount of moisture in the system.

Bergström (*6*), working with sodium linoleate in an aqueous medium in the presence of cupric ion, was able to add two moles of oxygen per mole of linoleate without appreciable polymerization taking place. He pointed out the desirability of the method in studying the structure of the oxidation products, for in the ordinary autoxidation of linoleate to the degree where four oxygen atoms have been absorbed marked polymerization occurs.

The behavior of antioxidants in an aqueous fat system as represented in baked goods is much different from that in pure fats. It is difficult to understand why so few of the recognized antioxidants have adequate carry-over in baked products. No satisfactory explanation has been made for the superior carry-over properties of butylhydroxyanisole. It appears that the aqueous fat systems offer a fertile field for research.

Stabilization of Fats

The use of antioxidants is the most practical and efficient method of preventing the development of rancidity in fats, although other methods of improving the stability of fats are generally well known.

During the processing of fats, unnecessary exposure to heat and light should be avoided, especially in the presence of oxygen. Care should be taken to avoid contact of the fat with metals that readily yield metallic ions to the fat, for most metallic ions act as pro-oxidants.

Hydrogenation increases the stability of fats by decreasing the polyunsaturated acids preferentially, and in some cases appears to regenerate antioxidants from the minor constituents of the oil.

In the field of packaging of fats, it is important to prevent the access of light and oxygen. Oxygen is usually excluded by vacuum packing or by the use of inert gas to displace the oxygen.

This subject of stabilization of fats was recently reviewed by Lundberg (*42*).

Literature Cited

(1) Allen, R. R., Jackson, A., and Kummerow, F. A., *J. Am. Oil Chemists' Soc.*, **26,** 395 (1949).
(2) Atherton, D., and Hilditch, T. P., *J. Chem. Soc.*, **1944,** 105.
(3) Bach, A., *Compt. rend.*, **124,** 951 (1897).
(4) Bailey, A. E., and Fisher, G. S., *Oil & Soap*, **23,** 14 (1946).
(5) Bergström, S., *Arkiv Kemi Mineral. Geol.*, **21A,** No. 14, 1 (1945); *Nature*, **156,** 717 (1945).
(6) Bergström, S., Blomstrand, R., and Laurell, S., *Acta Chem. Scand.*, **4,** 245 (1950).
(7) Bickford, W. G., Fisher, G. S., Kyame, L., and Swift, C. E., *J. Am. Oil Chemists' Soc.*, **25,** 254 (1948).
(8) Bickford, W. G., Krauczunas, P., and Wheeler, D. H., *Oil & Soap*, **19,** 23 (1942).
(9) Bolland, J. L., and Gee, G., *Trans. Faraday Soc.*, **42,** 244 (1946).
(10) Brekke, J., and MacKinney, G., *J. Am. Oil Chemists' Soc.*, **27,** 238 (1950).
(11) Brice, B. A., Swain, M. L., Herb, S. F., Nichols, P. L., Jr., and Riemenschneider, R. W., *Ibid.*, **29,** 279 (1952).
(12) Cannon, J. A., Zilch, K. T., Burk[illegible] S. C., and Dutton, H. J., *Ibid.*, **29,** 447 (1952).
(13) Chang, I., and Watts, B. M., *Food Research*, **15,** 313 (1950).
(14) Chang, S. S., and Kummerow, F. A., *J. Am. Oil Chemists' Soc.*, **30,** 251 (1953).
(15) Criegee, R., Pilz, H., and Flygare, H., *Ber.*, **72,** 1799 (1939).
(16) Dugan, L. R., Jr., Beadle, B. W., and Henick, A. S., *J. Am. Oil Chemists' Soc.*, **25,** 153 (1948).
(17) Dutton, H. J., Lancaster, C. R., Evans, C. D., and Cowan, J. C., *Ibid.*, **28,** 115 (1951).
(18) Engler, C., and Wild, W., *Ber.*, **30,** 1669 (1897).
(19) Farmer, E. H., *J. Soc. Chem. Ind.*, **66,** 86 (1947); *Rubber Chem. & Technol.*, **21,** 27 (1948).
(20) Farmer, E. H., *Trans. Faraday Soc.*, **38,** 340, 356 (1942).
(21) *Ibid.*, **42,** 233 (1946).
(22) Farmer, E. H., Koch, H. P., and Sutton, D. A., *J. Chem. Soc.*, **1943,** 541.
(23) Farmer, E. H., and Sundralingam, A., *Ibid.*, **1942,** 121.
(24) Farmer, E. H., and Sutton, D. A., *Ibid.*, **1943,** 119; **1946,** 10.
(25) Fugger, J., Cannon, J. A., Zilch, K. T., and Dutton, H. J., *J. Am. Oil Chemists' Soc.*, **28,** 285 (1951).
(26) Fugger, J., Zilch, K. T., Cannon, J. A., and Dutton, H. J., *J. Am. Chem. Soc.*, **73,** 2861 (1951).
(27) Gunstone, F. D., and Hilditch, T. P., *J. Chem. Soc.*, **1945,** 836.
(28) *Ibid.*, **1946,** 1022.
(29) Herb, S. F., and Riemenschneider, R. W., *Anal. Chem.*, **25,** 953 (1953).
(30) Hilditch, T. P., *J. Oil & Colour Chemists' Assoc.*, **30,** 1 (1947).
(31) Hilditch, T. P., *Nature*, **166,** 558 (1950).
(32) Hoch, H., and Lang, S., *Ber.*, **76,** 169 (1943).
(33) Holman, R. L., *Arch. Biochem.*, **15,** 403 (1947).
(34) Holman, R. T., and Elmer, O. C., *J. Am. Oil Chemists' Soc.*, **24,** 127 (1947).
(35) Kawahara, F. K., and Dutton, H. J., *Ibid.*, **29,** 372 (1952).
(36) Khan, N. A., Brown, J. B., and Deatherage, F. E., *Ibid.*, **28,** 105 (1951).
(37) Knight, H. B., Eddy, C. R., and Swern, D., *Ibid.*, **28,** 188 (1951).
(38) Lea, C. H., "Rancidity In Edible Fats," New York, Chemical Publishing Co., 1939.
(39) Lewis, W. R., and Quackenbush, F. W., *J. Am. Oil Chemists' Soc.*, **26,** 53 (1949).
(40) Lewis, W. R., Quackenbush, F. and DeVries, T., *Anal. Chem.*, **2[illegible]**, 762 (1949).

(41) Lips, H. J., *Food in Canada*, **12,** 9 (1952).
(42) Lundberg, W. O., Hormel Institute, University of Minnesota, *Pub.* **20** (1947).
(43) Lundberg, W. O., *Oleagineux*, **4,** 86 (1949).
) Lundberg, W. O., Chipault, J. R., and Hendrickson, M. J., *J. Am. Oil Chemists' Soc.*, **26,** 109 (1949).
(45) Max, R. A., and Deatherage, F. E., *Ibid.*, **28,** 110 (1951).
(46) Powers, P. O., *Ind. Eng. Chem.*, **41,** 304 (1949).
(47) Privett, O. S., and Lundberg, W. O., *J. Am. Oil Chemists' Soc.*, **28,** 313 (1951).
(48) Privett, O. S., Lundberg, W. O., Khan, N. A., Tolberg, W. E., and Wheeler, D. H., *Ibid.*, **30,** 61 (1953).
(49) Ross, J., Gebhart, A. I., and Gerecht, J. F., *J. Am. Chem. Soc.*, **68,** 1373 (1946).
(50) *Ibid.*, **71,** 282 (1949).
(51) Schepartz, A. I., and Daubert, B. F., *J. Am. Oil Chemists' Soc.*, **27,** 367 (1950).
(52) Sims, R. P. A., *Can. Chem. Proc. Ind.*, **35,** 125 (1951).
(53) Skellon, J. H., *J. Soc. Chem. Ind.*, **50,** 382T (1931).
(54) Stapf, R. J., and Daubert, B. F., *J. Am. Oil Chemists' Soc.*, **27,** 374 (1950).
(55) Stephens, H. N., *J. Am. Chem. Soc.*, **50,** 568 (1928).
(56) Stirton, A. J., Turer, J., and Riemenschneider, R. W., *Oil & Soap*, **22,** 81 (1945).
(57) Swain, M. L., and Brice, B. A., *J. Am. Oil Chemists' Soc.*, **26,** 272 (1949).
(58) Swern, D., Coleman, J. E., Knight, H. B., Ricciuti, C., Willits, C. O., and Eddy, C. R., *J. Am. Chem. Soc.*, **75,** 3135 (1953).
(59) Swern, D., Knight, H. B., Scanlan, J. T., and Ault, W. C., *Ibid.*, **67,** 1132 (1945).
(60) Swern, D., Scanlan, J. T., and Knight, H. B., *J. Am. Oil Chemists' Soc.*, **25,** 193 (1948).
(61) Swift, C. E., and Dollear, F. G., *Ibid.*, **25,** 52 (1948).
(62) Swift, C. E., O'Connor, R. T., Brown, L. E., and Dollear, F. G., *Ibid.*, **26,** 297 (1949).
(63) Wheeler, D. H., and Riemenschneider, R. W., *Oil & Soap*, **16,** 207 (1939).
(64) Willits, C. O., Ricciuti, C., Knight, H. B., and Swern, D., *Anal. Chem.*, **24,** 785 (1952).
(65) Willits, C. O., Ricciuti, C., Ogg, C. L., Morris, S. G., and Riemenschneider, R. W., *J. Am. Oil Chemists' Soc.*, **30,** 420 (1953).
(66) *Ibid.*, in press.