# Formation of Traces of Conjugated Tetraenoic and Trienoic Constituents in Autoxidized Linolenic and Linoleic Acids and Vegetable Oils During Alkali-Isomerization*

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## Introduction

THE occurrence of small proportions of arachidonic acid, a $C_{20}$ nonconjugated tetraenoic fatty acid, in animal fats is well known through the work of Ellis and Isbell (1), Brown and Dick (2), Hilditch and Longenecker (3), and others. Recently it has been shown that spectrophotometric methods are far more sensitive than the octabromide test for detecting this acid. The spectral characteristics of alkali-isomerized arachidonic acid have been studied by Mowry *et al.* (4), and by Beadle and Kraybill (5, 6). Beadle *et al.* (7) proposed the spectrophotometric method as a means of detecting animal fats in hydrogenated vegetable oil shortenings.

Brice and Swain (8) extended the sensitivity and accuracy of the spectrophotometric method by introducing a more transparent isomerization medium, correcting observed absorption for extraneous "background" absorption, and including a determination of trace conjugated constituents. In applying this method to the analyses of animal fats, selectively hydrogenated animal fats, and their soaps (9, 10), it was found that as little as 0.01% arachidonic acid should be detectable, with similar limits for linolenic and linoleic acids under favorable conditions and with even lower limits for traces of conjugated trienoic and tetraenoic constituents. Detailed criteria for determining the presence or absence of trace polyunsaturated fatty acid constituents are presented in reference (10).

During the study this refined method was applied to a few vegetable oils. Samples of perilla and soybean oils after alkali isomerization showed absorption maxima and inflections near 316 and 300 mμ that were indistinguishable from those shown by alkali-isomerized animal fats. On the basis of this spectroscopic evidence it was reported (8, 9) that these samples contained small proportions of a nonconjugated tetraenoic fatty acid constituent (1.3 and 0.13% for perilla oils, and 0.98% for soybean oil, in terms of arachidonic acid as a standard). Although chemical evidence for the presence of such an acid had never been reported, it was considered possible that the acid might nevertheless have been present and remained undetected because of the insensitivity of chemical methods, such as the octabromide test, in the presence of substantial proportions of linolenic acid.

A similar uncertainty exists in the reported detection by spectrophotometric analysis (9) of 0.19 to 0.54% linolenic acid in tobacco seed oil, which after alkali-isomerization exhibited absorption bands of a structure characteristic of linolenic acid. No chemical evidence has been presented for the presence of linolenic acid in oils of this type (11).

The present investigation was undertaken to clarify these points. The study was extended to a large number of vegetable oils and to freshly prepared and autoxidized linolenic and linoleic acids. Evidence was obtained that during the mild autoxidation of linolenic acid, oxidation products form which after alkali-isomerization produce absorption bands in the tetraenoic region similar to those produced by the arachidonic acid of animal fats. These products can be distinguished from arachidonic acid, however, by their conjugation on heating in neutral ethylene glycol. A similar situation exists with respect to autoxidized linoleic acid, in which bodies capable of conjugating on heating or on alkali-isomerization are present.

## Spectral Characteristics of Alkali-isomerized Vegetable Oils

Of the total of fifty or more commercial and noncommercial samples of vegetable oils spectrophotometrically analyzed, including representatives of the drying, semi-drying, and non-drying classes, approximately one-half yielded the tetraenoic bands on alkali-isomerization. The intensity of the bands in general was greatest in oils of the drying class although some samples of these oils were completely free of tetraenoic absorption after alkali-isomerization, and some samples of semi- and non-drying oils yielded tetraenoic bands of low intensity. Table I shows the spectrophotometric analyses of a few representative oils, with the tetraenoic absorption, suitably corrected for background, calculated as per cent arachidonic acid. The analytical method used, described in references (8, 9, 10), involved measurement of the ultra-violet absorption of the samples before and after heating for 30 minutes at 180° C. in 11% KOH-glycerol. Spectra of representative alkali-isomerized oils and of the alkali-isomerized arachidonic acid[1] used as a standard for the calculation of the tetraenoic constituent are shown in Figure 1. The similarities in spectral characteristics in the 300-320 mμ region of the isomerized oils and arachidonic acid are clearly apparent and leave little doubt that the chromophore responsibile for the absorption in the oils is essentially identical with that in the acid.

Although the presence of a system of four conjugated double bonds in the isomerized oils was established with reasonable certainty by the characteristics of the observed absorption, the spectroscopic findings provided no information regarding the chemical identity of either the compound containing the chromophoric group or its non-absorbing precursor. That the latter is of fatty acid nature, however, may be

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[1] The authors are indebted to J. B. Brown of Ohio State University for the sample of methyl arachidonate which was used as a standard to establish the constants for alkali-isomerized arachidonic acid. The ester, prepared by Mrs. Betty Orians in Professor Brown's laboratory, had an iodine number of 321 (theoretical value, 319) and an octabromide number of 100.6.

TABLE I

Spectrophotometric Analyses of Vegetable Oils

| No. | Oil | Description | Nonconjugated Acids, % | | | Conjugated Acids, % | | |
|---|---|---|---|---|---|---|---|---|
| | | | Tetraenoic as Arachidonic [b] | Linolenic | Linoleic | Tetraenoic [b] | Trienoic [b] | Dienoic |
| 1 | Perilla | Refined | 1.3 | 55.6 | 13.0 | 0.02 | 0.07 | 2.2 |
| 2 | Perilla | Extracted | 0.13 | 53.9 | 16.9 | .002 | .001 | 0.19 |
| 3 | Linseed | Raw | 0.41 | 45.6 | 18.0 | .007 | .005 | 0.58 |
| 4 | Linseed | Crude | 1.1 | 39.3 | 14.7 | .01 | .02 | 2.8 |
| 5 | Linseed | Refined | 0.12 | 50.2 | 11.7 | .002 | .001 | 0.60 |
| 6 | Soybean | Cold Pressed | 1.0 | 4.1 | 43.3 | .000 | .01 | 2.6 |
| 7 | Soybean | Refined | 0.32 | 6.5 | 52.4 | .000 | .001 | 0.70 |
| 8 | Tobacco Seed | Extracted | 0.02 | 0.53 [a] | 70.0 | .000 | 0.004 | 0.16 |
| 9 | Corn | Crude | .00 | .38 [a] | 57.2 | .000 | .01 | .36 |
| 10 | Cottonseed | Refined | .01 | .79 [a] | 50.5 | .004 | .12 | .32 |
| 11 | Rape | Refined | .10 | 6.8 | 14.4 | ...... | ...... | ...... |
| 12 | Tea Seed | Refined | .03 | 1.1 [a] | 7.5 | .000 | .01 | 1.0 |
| 13 | Olive | Commercial | .01 | .60 [a] | 9.1 | .001 | .012 | .29 |

[a] Calculated as linolenic acid; absorption characteristic of triene conjugation was corrected for extraneous background absorption (8).
[b] After correction for extraneous background absorption.

deduced from its behavior toward the chemical and physical treatments employed in preparing pure linolenic acid from perilla oil, spectroscopic examination of the isomerized concentrates being used to follow the constituent through the various processes. Thus the constituent was found in the freed fatty acids of the oil, was concentrated in the more unsaturated fraction of the acids on low temperature crystallization, appeared in the linolenic acid recovered by bromination-debromination techniques, and was removed from the methyl linolenate and linolenic acids obtained as final products only by repeated distillations or crystallizations. This behavior was compatible with that to be expected of a non-conjugated tetraenoic fatty acid, but similar findings might be expected if the tetraenoic conjugation was derived from an oxidation product continuously formed from linolenic acid during its concentration. Thus O'Connor *et al.* (12) had shown that unless oxygen was rigorously excluded during every step of the process in the preparation of pure linolenic acid, the spectrum of the acid, unisomerized but subjected to distillation at 182-183°, contained bands characteristic of tetraenoic conjugation. Tetraene conjugation was completely absent from the spectrum of linolenic acid prepared in the strict absence of oxygen. Likewise the analogous triene conjugation encountered in the spectrum of distilled linoleic acid, which had been exposed to oxygen during its purification, failed to appear if such exposure was avoided. Similarly, Mitchell *et al.* (13) found characteristic tetraenoic bands in the spectra of ethyl linolenate and linolenic acid-containing oils and characteristic triene bands in methyl linoleate and linoleic acid-containing oils when these materials were subjected to the action of bleaching earths following oxidation by blowing with air. They attributed the formation of the observed conjugation to the dehydrative action of the bleaching earth on oxidation products of the polyunsaturated acids and postulated several possible structures for these products.

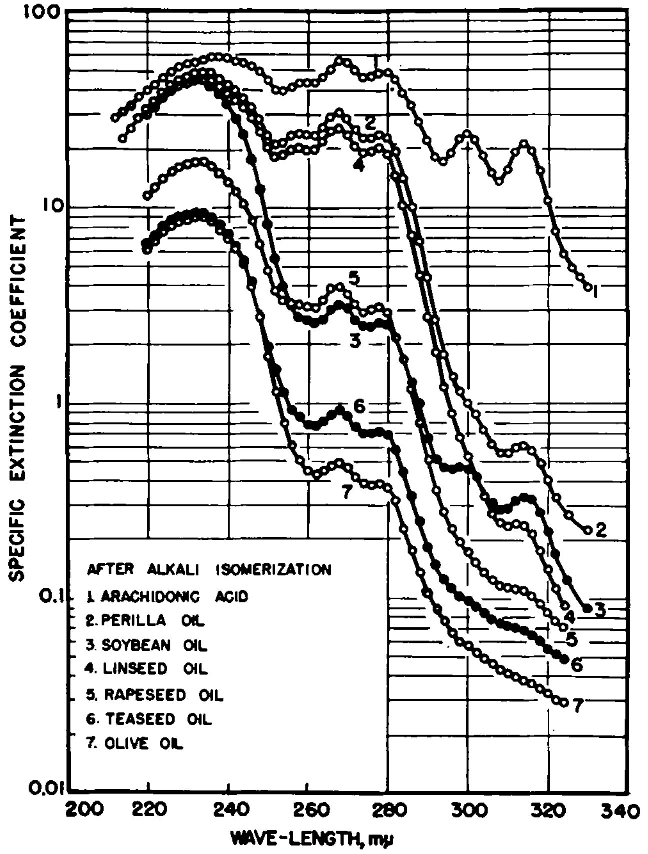


Fig. 1. Ultra-violet absorption spectra of vegetable oils and of arachidonic acid after isomerization in KOH-glycerol 30 minutes at 180°C.

## Effect of Alkali-isomerization and Heat on Autoxidized Linolenic Acid

Since dehydrative conditions exist during the alkali-isomerization process, it seemed desirable to determine whether the formation of tetraenoic conjugation in vegetable oils during the process was related to their state of oxidation before isomerization. The possibility of the formation of tetraenoic conjugation by oxidation of the sample during the isomerization process itself was precluded by the absence of tetraenoic bands from the spectra of isomerized linolenic acids and esters of high degrees of purity.

Small samples of highly purified linolenic acids and esters (prepared under nitrogen, but with some oxygen apparently present), originally used as standards for the calibration of the spectrophotometric method, were available in an autoxidized state resulting from approximately two years' storage in the dark at approximately 10°C. in the presence of air. These acids and esters appeared to offer suitable material for a determination of the effect of autoxidation on the spectrum obtained on alkali-isomerization of linolenic acid and were therefore re-analyzed. The spectrophotometric analyses of a series of linolenates and linolenic acid concentrates before and after such autoxidation are shown in Table II. Spectra of a typical linolenic acid sample before and after alkali-isomerization in both its original and autoxidized forms are shown in Figure 2. The most marked changes in the spectra resulting from oxidation of the samples are the great increase in preformed diene conjugation probably indicating the presence of conjugated hydroperoxides, and the development of tetraenoic conjugation equivalent to that produced by about 5% arachidonic acid following alkali-isomerization.

Tetraenoic conjugation of approximately equal amount, accompanied by a loss of preformed diene, is produced on subjecting the oxidized sample to a heat treatment equivalent to that required for alkali-isomerization, with neutral ethylene glycol substituted for the alkaline glycerol reagent as a solvent medium. This behavior, which indicates that no alkali-induced shift of double bonds is involved, is illustrated in curve 2h of Figure 2. In contrast, arachidonic acid, in which a system of four non-conjugated double bonds exists, fails to develop any appreciable tetraenoic conjugation on heating.

The difference in the behavior of oxidized linolenic acid and of arachidonic acid on heating in neutral ethylene glycol suggested that this simple heating process might be applied as a criterion to samples yielding tetraenoic conjugation of questionable origin on isomerization in order to determine whether such samples contain a non-conjugated tetraenoic acid of the arachidonic acid type or merely oxidation products of linolenic acid. The fatty acid fractions of a number of the vegetable oils previously analyzed were accordingly heated in neutral ethylene glycol. The amount of tetraenoic conjugation formed as a result of the heat treatment was in every case equal to or greater than that formed on alkali-isomerization, and it may therefore be concluded that an oxidation product of linolenic acid rather than a non-conjugated tetraenoic acid is the precursor of at least the major portion of the tetraenoic conjugation observed in vegetable oils. The spectra of the soybean oil fatty acids shown in Figure 4 are illustrative of the relative degrees of development of tetraenoic conjugation in vegetable oils on heating and on alkali isomerization. The spectra of the beef tallow fatty acids shown in the same figure illustrate the difference in behavior of fats containing arachidonic acid. Full development of the tetraenoic bands in beef fat is dependent on the presence of alkali; the slight enhancement of the preformed tetraenoic absorption on heating is undoubtedly due in this case, as in the vegetable oils, to traces of oxidized linolenic acid.

It is of interest to note that the preformed conjugated diene of beef fat also differs from that of the oxidized polyunsaturated acids and that of the oxidized vegetable oil fatty acids in showing no significant decrease on heating. The stability of the conjugated diene of beef fat has been observed in a number of samples, including concentrates of the more unsaturated acids of the fat containing as much as 4 to 5% preformed conjugated diene, probably indicating that a conjugated linoleic acid rather than a conjugated hydroperoxide is responsible for most of the conjugated diene absorption regularly observed in tallow.

### Effect of Alkali-isomerization and Heat on Autoxidized Linoleic Acid

The formation of conjugated tetraenoic absorption on alkali-isomerization of samples containing oxidized linolenates suggested that triene conjugation might be similarly formed on isomerization of oxidized linoleates. Samples of linoleic acid and its ester which had originally possessed a high state of purity and which had been exposed to the same conditions of autoxidation as those obtaining for the linolenates were therefore re-analyzed. Analytical data for these samples are given in Table II. Spectra for a typical sample of linoleic acid before and after alkali-isomerization in its original and autoxidized states are shown in Figure 3. As in the case of oxidized linolenic acid, a great increase in preformed

TABLE II

Comparison of Spectrophotometric Analyses of Purified Polyunsaturated Acid Preparations and Concentrates Before and After Autoxidation; and the Effect of Heating in Neutral Ethylene Glycol (last two columns)

| No. | Sample | Autoxidation | I. V.[a] | P. V.[b] | Nonconjugated Acids, %: Linoleic | Nonconjugated Acids, %: Linolenic | Nonconjugated Acids, %: Tetraenoic [c] | Conjugated Acids, %: Dienoic | Conjugated Acids, %: Trienoic | Conjugated Acids, %: Tetraenoic | After Heating: Apparent [c] Tetraenoic Acid | After Heating: Apparent [d] Linolenic Acid |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Linolenic Acid | Before | 273.5 | ...... | 0.00 | 99.0 | 0.17 | 1.5 | 0.00 | 0.004 | | |
| | | After | ...... | 364 | 0.00 | 68.8 | 4.8 | 7.4 | 0.12 | 0.00 | 5.3 | ...... |
| 2 | Methyl Linolenate | Before | 260.0 | ...... | 0.00 | 95.0 | 0.03 | 2.0 | 0.00 | 0.001 | | |
| | | After | ...... | 659 | 4.0 | 73.5 | 3.2 | 3.6 | 0.02 | 0.003 | ...... | ...... |
| 3 | Linolenic Acid Concentrate | Before | 236.6 | ...... | 15.8 | 62.9 | 0.34 | 1.5 | 0.13 | 0.08 | | |
| | | After | ...... | 420 | 19.4 | 63.3 | 1.7 | 2.0 | 0.08 | 0.03 | 2.7 | 0.45 |
| 4 | Ethyl Linolenate Concentrate | Before | ...... | ...... | 11.1 | 60.2 | 0.22 | 1.3 | 0.11 | 0.10 | | |
| | | After | ...... | ...... | 7.3 | 51.9 | 3.8 | 10.0 | 0.23 | 0.00 | 3.3 | ...... |
| 5 | Linoleic Acid | Before | 181.9 | ...... | 98.0 | 0.00 | 0.00 | 1.2 | 0.01 | 0.00 | | |
| | | After | ...... | 102 | 83.1 | 0.26 [d] | 0.00 | 7.7 | 0.00 | 0.00 | 0.08 | 8.2 |
| 6 | Methyl Linoleate | Before | 175.0 | ...... | 96.0 | 0.00 | 0.00 | 0.43 | 0.03 | 0.001 | | |
| | | After | ...... | 2053 | 19.0 | 0.68 [d] | 0.00 | 13.0 | 0.00 | 0.00 | ...... | ...... |
| 7 | Ethyl Linoleate | Before | 165.7 | ...... | 89.2 | 0.00 | 0.00 | 2.6 | 0.00 | 0.00 | | |
| | | After | ...... | 2250 | 9.8 | 1.1 [d] | 0.00 | 18.9 | 0.00 | 0.00 | ...... | ...... |
| 8 | Ethyl Linoleate Concentrate | Before | 151.0 | ...... | 75.5 | 1.3 [d] | 0.00 | 0.94 | 0.02 | 0.00 | | |
| | | After | ...... | 206 | 63.2 | 1.5 [d] | 0.00 | 3.2 | 0.00 | 0.00 | 0.0 | 3.4 |

[a] Iodine value, Wijs one-half hour.

[b] Peroxide value, milliequivalent of active oxygen per 1,000 g. of sample.

[c] Calculated as apparent arachidonic acid. The actual proportion of conjugated tetraene in the treated sample is approximately 0.1 these values.

[d] Calculated as apparent linolenic acid. The actual proportion of conjugated triene in the treated sample is approximately 0.4 these values.

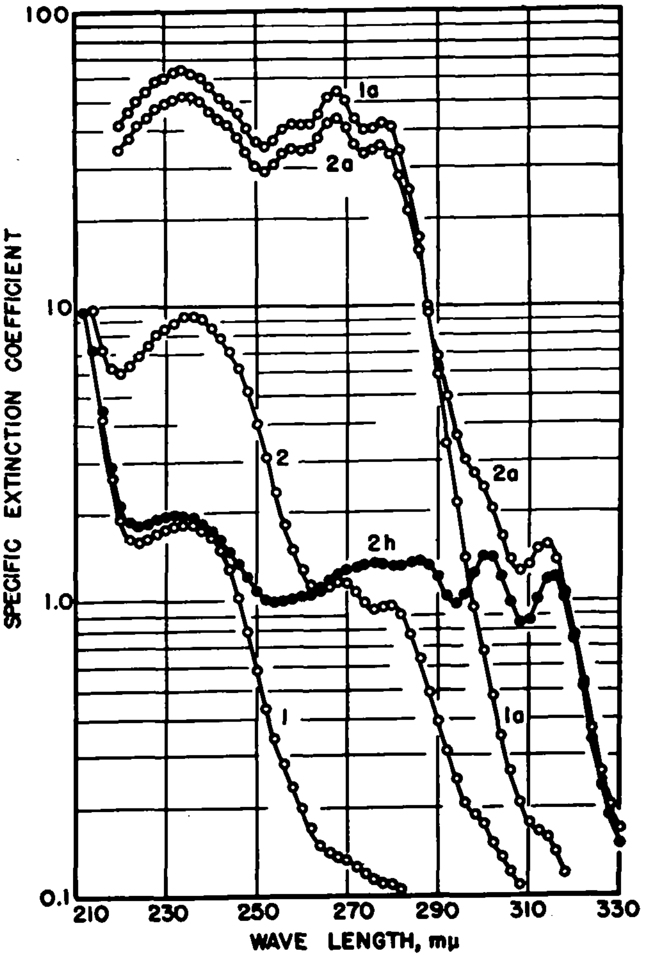


Fig. 2. Ultra-violet absorption spectra of freshly prepared linolenic acid, before (1) and after (1a) alkali isomerization; of autoxidized linolenic acid, before (2) and after (2a) alkali isomerization, and after heating in ethylene glycol (2h).

diene conjugation is apparent, but no large increase in conjugated triene absorption of the alkali-isomerized product obtained from the oxidized acid is evident if due allowance is made for the contribution of background absorption. Heating the oxidized acid in neutral ethylene glycol (for 30 minutes at 180°C.), however, leads to the formation of large amounts of conjugated triene, apparently at the expense of the preformed diene, which suffers a marked decrease. Once formed, such triene conjugation shows the same relative stability toward heating in the alkaline isomerization medium as do the known eleostearates (8), as is illustrated in curve 2ha, Figure 3, for oxidized linoleic acid which has been isomerized after a preliminary heating. The appearance of tetraenoic absorption in this curve is interesting in that it demonstrates the possibility of deriving four double-bond conjugation from linoleic acid and hence affords a possible explanation for the tetraenoic conjugation found in isomerized vegetable oils of low linolenic acid content.

Although the triene absorption formed on alkali isomerization of oxidized linoleic acid is of low intensity, it probably accounts for at least a share of the triene conjugation usually encountered in isomerized peanut, corn, cottonseed, and other similar oils of high linoleic acid content. Hilditch and Shrivastava (11) have recently questioned the propriety of calculating as linolenic acid the triene conjugation of these isomerized oils since linolenic acid could not be demonstrated as present by chemical means even on concentration of the more highly unsaturated fractions. It would seem that in these cases, as in the case of the tetraenoic conjugation of vegetable oils, such absorption should be disregarded in the usual analysis of common vegetable oils and be considered only in special studies where they may have significance. In questionable cases the development of equivalent or greater amounts of absorption as a result of heating in neutral ethylene glycol indicates that the precursor is an oxidation product, and failure of the bands to develop on heating in neutral medium indicates the presence of a non-conjugated acid. The heating in neutral ethylene glycol must be carried out on the free fatty acid fraction of oils and esters since considerable polymerization or condensation appears to occur if the intact oils or esters are heated, even though a medium such as glycol ether, which has greater solvent power for oils, is substituted for ethylene glycol.

## Discussion

The true nature of the oxidation products of the polyunsaturated acids which on alkali-isomerization yield conjugated unsaturation of a higher degree

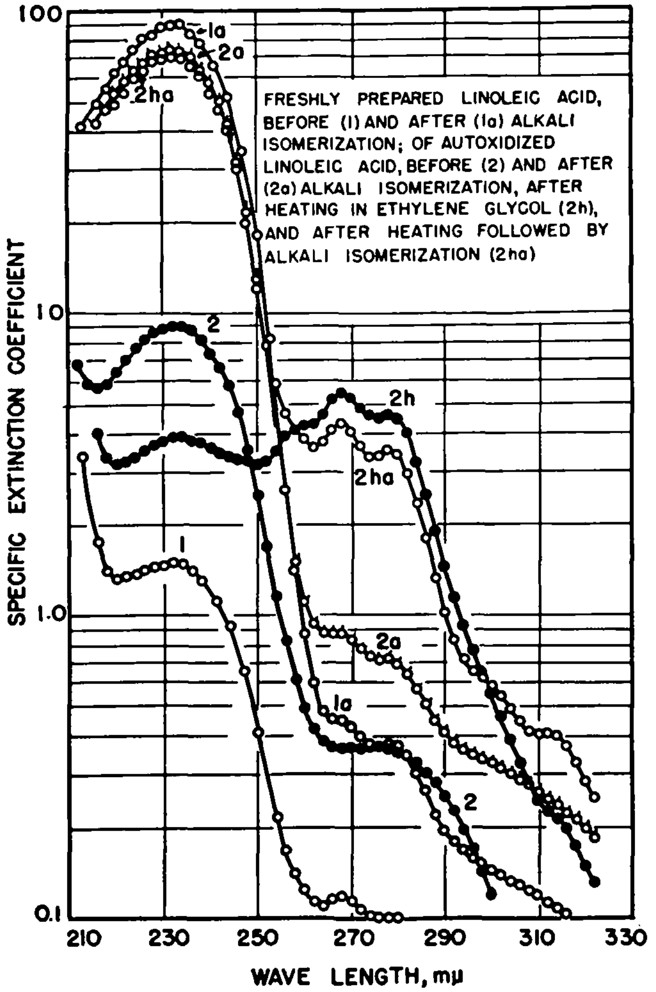


Fig. 3. Ultra-violet absorption spectra of freshly prepared linoleic acid, before (1) and after (1a) alkali isomerization; of autoxidized linoleic acid, before (2) and after (2a) alkali isomerization, after heating in ethylene glycol (2h), and after heating followed by alkali isomerization (2ha).

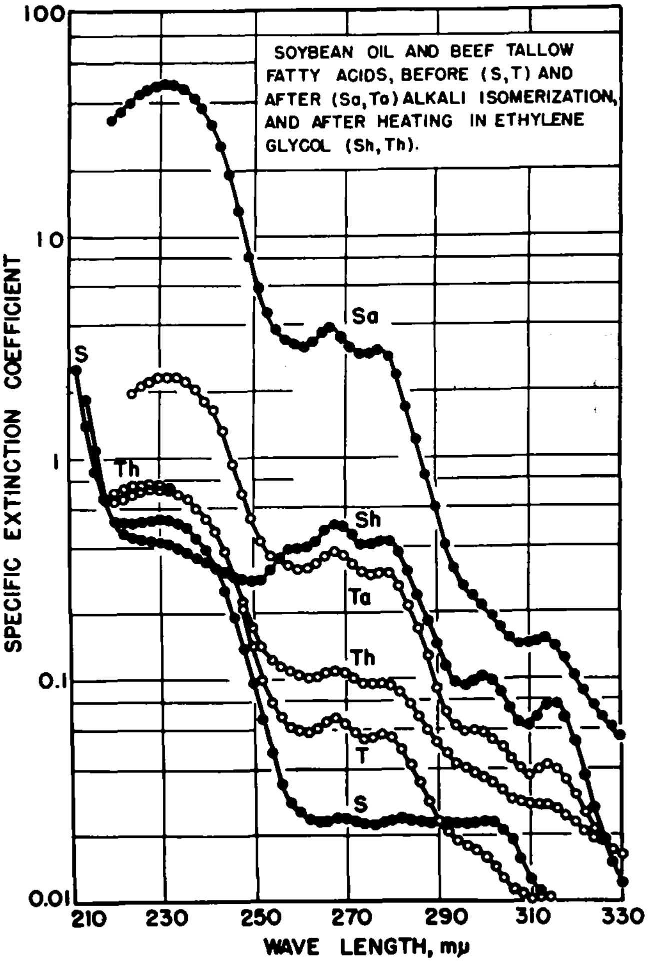
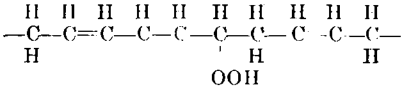


Fig. 4. Ultra-violet absorption spectra of soybean oil and beef tallow fatty acids, before (S, T) and after (Sa, Ta) alkali isomerization, and after heating in ethylene glycol (Sh, Th).

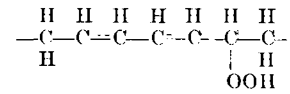
than that possessed by the parent acid cannot be fully determined without isolation and systematic study of the primary oxidation products of the fatty acids. It seems reasonable to assume, however, that dehydration of oxygenated structures at the high temperature required for the isomerization treatment is chiefly responsible for the introduction of the new double bond. Thus two of the structures for the monohydroperoxide of linolenic acid postulated by Farmer *et al.* (14) in which the arrangement of the double bond is as in (I) could, after



$$\begin{array}{cccccccccc} H & H & H & H & H & H & H & H & H & H \\ -C- & C= & C- & C- & C- & C- & C- & C- & C- & C- \\ H & & & & & & H & & & H \\ & & & & & & OOH & & & \end{array} \qquad I$$

hydrolysis or decomposition of the peroxide to the corresponding hydroxy acid, lose water with relative ease, since the hydrogen atoms on the carbon atom adjacent to the hydroxyl group are activated by the neighboring double bond. Dehydration in this case would yield a conjugated tetraenoic system and be accompanied by the loss of conjugated diene observed on heating. If this view is correct, the conjugated fatty acid produced would be parinaric acid or its isomer.

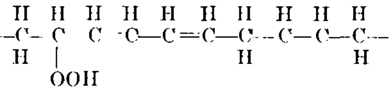
A somewhat different system exists in linoleic acid, in which structures such as (II) are postulated for



$$\begin{array}{ccccccc} H & H & H & H & H & H & H \\ -C- & C= & C- & C= & C- & C- & C- \\ H & & & & & | & H \\ & & & & & OOH & \end{array} \qquad II$$

the conjugated monohydroperoxides (15), since in this case the hydrogen atoms on the carbon atom adjacent to that bearing the peroxide group are unactivated by a neighboring double bond. A difference in the rates of formation of triene conjugation from oxidized linoleic acid, and tetraenoic conjugation from oxidized linolenic acid, is actually detectable experimentally. Thus the maximum tetraenoic absorption obtainable from oxidized linolenic acid is realized after 10 minutes' heating at 180°C., whereas the triene absorption derivable from oxidized linoleic acid is still increasing after 45 minutes' heating. This may account for the observed difference in the amounts of triene conjugation formed on heating oxidized linoleic acid in the presence and absence of alkali since a slow rate of dehydration would result in a predominance of peroxide breakdown to unsaturated carbonyl compounds in the presence of alkali. Some decomposition of the peroxide to unsaturated carbonyl undoubtedly occurs on heating in neutral ethylene glycol since a smooth peak with a maximum in the 270-280 mμ region appears to underlie the characteristic triene absorption. The bright yellow color usually obtained on treating oxidized unsaturated acids with alkali, apparently resulting from condensations of unsaturated carbonyl compounds (16), is markedly diminished in intensity in both oxidized linoleic and linolenic acids after a preliminary heating in neutral ethylene glycol, indicating that unsaturated carbonyl bodies or their precursors have been largely destroyed.

Although the proposed formulas for the conjugated hydroperoxides offer reasonable starting structures from which conjugation of the observed degrees can be obtained, a number of observations cannot be readily explained. For instance, one would expect that if structure II is capable of yielding triene conjugation in the case of linoleic acid, the similar structure III, postulated for two forms of linolenic



$$\begin{array}{cccccccccc} H & H & H & H & H & H & H & H & H & H \\ -C- & C & C & -C- & C= & C- & C- & C- & C- & C- \\ H & | & & & & & H & & H & \\ & OOH & & & & & & & & \end{array} \qquad III$$

acid monohydroperoxide, should yield triene conjugation with equal ease. However, the spectrum of heated oxidized linolenic acid shows little evidence for the formation of tetraene unsaturation. This may indicate that the forms of linolenic acid monohydroperoxide which yield triene conjugation on heating are favored. One would similarly expect that oleic acid hydroperoxide would yield diene conjugation on heating, but no evidence for such a mode of decomposition has been obtained with naturally autoxidized samples having peroxide numbers of 100 M. E. or more. Swift *et al.* (17) have recently shown that the thermal decomposition of methyl hydroperoxide oleate is accompanied by considerable chain scission, yielding unsaturated aldehydes as decomposition products.

Although the formation of conjugated polyunsaturation from oxidized polyunsaturated fatty acids during the alkali-isomerization treatment has some effect on the analytical results obtained by the spectrophotometric method, the errors introduced from this source in the analysis of ordinary samples are undoubtedly insignificant. Thus the amounts of oxidized linolenate likely to be present in animal fats are so small that their contribution to the total observed tetraenoic absorption after isomerization could hardly affect the value obtained for arachidonic acid. In the ordinary analysis of vegetable oils high in linolenic acid, observed tetraenoic conjugation after isomerization may be simply discounted, except as an index of the state of oxidation of the oil.

Fortunately, the amount of triene conjugation realized for even relatively large amounts of oxidized linoleic acid on alkali isomerization is low and contributes only small errors to the amounts of spectrophotometrically determined linolenic acid. The development of these bands is probably of analytical significance only in the analysis of pure preparations of polyunsaturated acids and their compounds, where it may erroneously indicate the presence of linolenic or a tetraenoic acid. The more highly unsaturated fractions obtained by crystallization or distillation techniques may similarly be in error when spectrophotometrically analyzed. In these cases some evaluation of the amount of conjugated absorption contributed by the oxidized components may be obtained by observing the degree of development of the bands on heating in the absence of alkali.

No interference from anomalous tetraenoic conjugation, arising from oxidized constituents of vegetable oils on alkali-isomerixation, is to be expected in the previously cited test of Beadle *et al.* (7) for the detection of lard in admixture with hydrogenated vegetable oil shortenings since hydrogenation should completely destroy such constituents. In compounded shortenings containing unhydrogenated or mildly hydrogenated vegetable oils, some measure of the amounts of tetraenoic conjugation contributed by the vegetable oil fraction may be obtained by subjecting the fatty acids of the sample to the heating test.

While the findings reported here are of only minor importance in the everyday analysis of vegetable oils, they may be of considerable significance in special studies, for example on autoxidation behavior, stability, and flavor constituents.

## Summary

It has been shown that the low-intensity absorption bands characteristic of conjugated tetraenoic and trienoic fatty acids frequently encountered in the ultra-violet spectra of alkali-isomerized vegetable oils prepared by ordinary commercial or laboratory extraction techniques have their probable origin in oxidation products of linolenic and linoleic acid, respectively. Similar bands are found in the spectra of mildly autoxidized preparations of pure linolenic and linoleic acids after either alkali-isomerization or heating at 180°C. in neutral ethylene glycol.

Tetraenoic and trienoic conjugation formed from oxidation products of linolenic and linoleic acids during alkali-isomerization can be differentiated from the tetraenoic and trienoic conjugation produced by alkali-isomerization of arachidonic and linolenic acids, respectively, by spectrophotometric examination of the sample after heating in neutral ethylene glycol. Essentially equal amounts of conjugation are formed from the fatty acid oxidation products on heating and on alkali-isomerization while no conjugation is obtained from arachidonic and linolenic acids on heating in the absence of alkali.

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# Report of the Refining Committee 1948-1949

THE activities of the Refining Committee during the past year have been largely concerned with a further investigation of the centrifuge method as applied to degummed soybean oil and an attempt to improve the results by the standard A.O.C.S. Refining Method on degummed soybean oil. The different projects may be summarized as follows:

At the New Orleans meeting it was suggested that a demonstration of the centrifuge method of refining be scheduled and that those members of the Refining Committee who were interested either be present or send representatives. In August a meeting was held in the Hammond laboratories of Lever Brothers Company, at which the centrifuge method was demonstrated by C. J. Carmack. Attendance at this meeting was excellent, and those individuals present made a number of constructive suggestions.

In October the Northern Regional Laboratory sent out a series of four degummed soybean oils, two of the expeller type and two of the solvent extracted type for collaborative refining by the members of the committee. A total of 11 laboratories reported on this collaborative work, and the general results were about the same as those previously observed. It was found, however, that many of the members had considerable difficulty in refining the degummed expeller oils by the standard A.O.C.S. Method, which was used as a comparison with the centrifuge results.

At the New Orleans meeting Mr. Mays had reported on a modification of the centrifuge method