

[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL CHEMISTRY OF THE PURDUE UNIVERSITY AGRICULTURAL EXPERIMENT STATION]

## Formation of Conjugated Material during Bleaching of Vegetable Oils<sup>1,2</sup>

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During the course of an investigation of the ultraviolet absorption spectra of vegetable oils, it was found that bleaching with activated fuller's earth causes the development of absorption bands. These bands are thought to be due to the formation of conjugated systems by oxidation and dehydration of linoleic and linolenic esters and glycerides. The changes in the spectra are small in terms of absolute values, but they are very definite and measurable quantities. It is estimated that in most of the commercially bleached oils which were examined, the conjugated material occurred to the extent of from 0.1 to 0.2%. Linseed, soybean, corn, and cottonseed oils were examined as well as the esters of linoleic and linolenic acids. Bleaching with fuller's earth is almost universal in fat and vegetable oil processing; it was thus of interest to discover the cause of these changes in the spectra.

### Experimental

**Instrument and Methods.**—All of the absorption data were obtained from measurements made with a photoelectric spectrophotometer, similar in design to that of Hogness, *et al.*,<sup>3</sup> employing a Hilger double monochromator with crystal quartz optics. A Munch<sup>4</sup> hydrogen discharge tube with a fused quartz window was used as the source of ultraviolet radiation. Solutions of the oils and fatty acid esters were made in "iso-octane" (2,2,4-trimethylpentane) which was distilled over potassium hydroxide before use. Dilutions were made, if necessary, to maintain the value of  $\log_{10} I_0/I$  between 0.200 and 0.800. Absorption cells 1 cm. in length with matched crystalline quartz end plates were used.

The absorption curves are plotted using the value of the specific absorption coefficient on the ordinate and the wave length in Å. units on the abscissa.

$$\text{Specific } \alpha = \frac{\log_{10} I_0/I}{cl}$$

$\alpha$  = absorption coefficient

$I_0$  = intensity of radiation transmitted by the solvent

$I$  = intensity of radiation transmitted by the solution

$c$  = concentration of solute in g. per 1000 ml.

$l$  = length in cm. of solution through which the light passes.

(1) Presented before the American Oil Chemists Society at Chicago, Oct. 8-10, 1941.

(2) From a thesis submitted to the Graduate Faculty of Purdue University by J. H. Mitchell, Jr., in partial fulfillment of the requirements for the degree of Doctor of Philosophy, June, 1941.

(3) Hogness, Zscheile and Sidwell, *J. Phys. Chem.*, **41**, 379 (1937).

(4) Munch, *This Journal*, **57**, 1863 (1935).

### Ultraviolet Absorption Spectra of Vegetable Oils at Various Stages of the Commercial Processing Treatment.

—The ultraviolet absorption spectra of linseed oil, soybean oil, corn oil, and cottonseed oil were obtained at various stages in the refining process: in the raw state, after alkali refining, after bleaching, and after deodorizing. In each case, the samples were produced from the same lot of raw oil.

In Fig. 1 the absorption spectra of several samples of linseed oil are shown. Bleaching caused a relatively large increase in the absorption coefficients, and three maxima are present at 2680, 3000 and 3160 Å. Curves 1, 2, and 3 represent different samples of raw linseed oil, while curves 1A and 2A represent bleached oil corresponding to 1 and 2. Bleached linseed oil, curve 4A, after being passed through a column of silicate adsorbent<sup>5</sup> gave an oil with the absorption curve 4B, which shows a decrease in height at 2680 Å. but not at 3000 or 3160 Å. This is an indication that at least two substances are responsible for the absorption. The raw linseed oil of this series, curve 4, after being refined with the special silicate adsorbent, gave the absorption curve 4C. Here, the lower absorption throughout the curve is probably due to the removal of certain non-fat constituents.

A series for corn oil and another for soybean oil shows, Fig. 2, the relatively high absorption found in the bleached samples. Curve 1, in each series, represents the crude oil; 2, alkali refined oil; 3, bleached oil; and 4, deodorized oil. The bands at 3000 Å and 3160 Å. are much lower in soybean than in linseed oil and are scarcely perceptible in corn oil. These bands were found to be associated with the presence of linolenic acid while the band with a maximum at 2680 Å. was found to be associated with the presence of linoleic acid.

**Preparation of Linoleic and Linolenic Esters.**—In order to study the effect of bleaching on linoleic and linolenic acids, their esters were prepared.

**Tetrabromostearic Acid.**—Tetrabromostearic acid was prepared by bromination,<sup>6</sup> in petroleum ether at 0°, of the fatty acids of corn oil. After washing with petroleum ether, the tetrabromide was dissolved in hot, high-boiling (98°) petroleum ether and a small quantity of Norite was added. After removal of the charcoal by filtration through an electrically heated Büchner funnel, the tetrabromide was recrystallized three times more from high boiling petroleum ether. The final product was snow white and had a melting point of 115.5°, measured with a calibrated total-immersion thermometer.

**Debromination of the Tetrabromide.**—The tetrabromide (100 g.) was debrominated<sup>6</sup> by adding it gradually to 80 g. of zinc dust and 300 cc. of hot absolute ethyl alcohol. About 350 cc. of 7.5 *N* absolute ethyl alcoholic hydrochloric acid was added gradually to the refluxing mixture, and

(5) Kraybill, Thornton and Eldridge, *Ind. Eng. Chem.*, **32**, 1138 (1940).

(6) Rollett, *Z. physiol. Chem.*, **62**, 410 (1909).

the boiling was continued for about three hours to ensure complete esterification. Methyl alcohol was substituted for ethyl alcohol in preparation of methyl esters.

The ester was removed from the alcoholic solution by extraction with petroleum ether. The ether solution was washed with water and dried over anhydrous sodium sulfate. After removal of the ether *in vacuo*, the ester was distilled at 1 mm. pressure. The water-clear ethyl linoleate had an iodine number of 163.2, theory 164.4.

**Hexabromostearic Acid.**—Hexabromostearic acid was prepared by bromination<sup>7</sup> of the fatty acids of linseed oil in ethyl ether at  $-10^{\circ}$ . The hexabromide was washed with large quantities of ethyl ether on a Büchner funnel and was then dissolved in boiling xylene. A small quantity of Norite was added to the solution and was removed by filtration through an electrically heated Büchner funnel. After recrystallization three times more from boiling xylene and washing with ethyl ether, the snow white product had a melting point of  $185.5^{\circ}$ , obtained with a calibrated total-immersion thermometer.

**Debromination of the Hexabromide.**—The hexabromide (100 g.) was debrominated<sup>7,8</sup> by adding it to 100 g. of zinc dust in 500 cc. of absolute ethyl alcohol. Five hundred cubic centimeters of alcoholic hydrochloric acid was added from a dropping funnel to the boiling mixture. Extraction and distillation were carried out as described for linoleic esters. The water-clear product had an iodine number of 247.9, theory 248.8, and an acid number of zero.

**Absorption Curves of the Esters after Fuller's Earth Treatment.**—Small quantities of the unsaturated esters, prepared as described before, and also of esters obtained from other sources, were treated with fuller's earth under conditions comparable to those used in bleaching the vegetable oils which were examined. Four per cent. of earth was used at a temperature of  $65^{\circ}$  for one hour.

Figure 3 shows curves for the treated and non-treated esters. The curve for "bleached" ethyl linolenate is very similar to that obtained for bleached linseed oil, which contains about 50% of this acid. The absence of a band at  $2325 \text{ \AA}$ . indicates that no systems of diene conjugation were formed during the debromination in the preparation of the esters.

(7) Rollett, *Z. physiol. Chem.*, **62**, 422 (1909).

(8) Norris, Kass and Burr, *Oil and Soap*, **XVII**, 123 (1940).

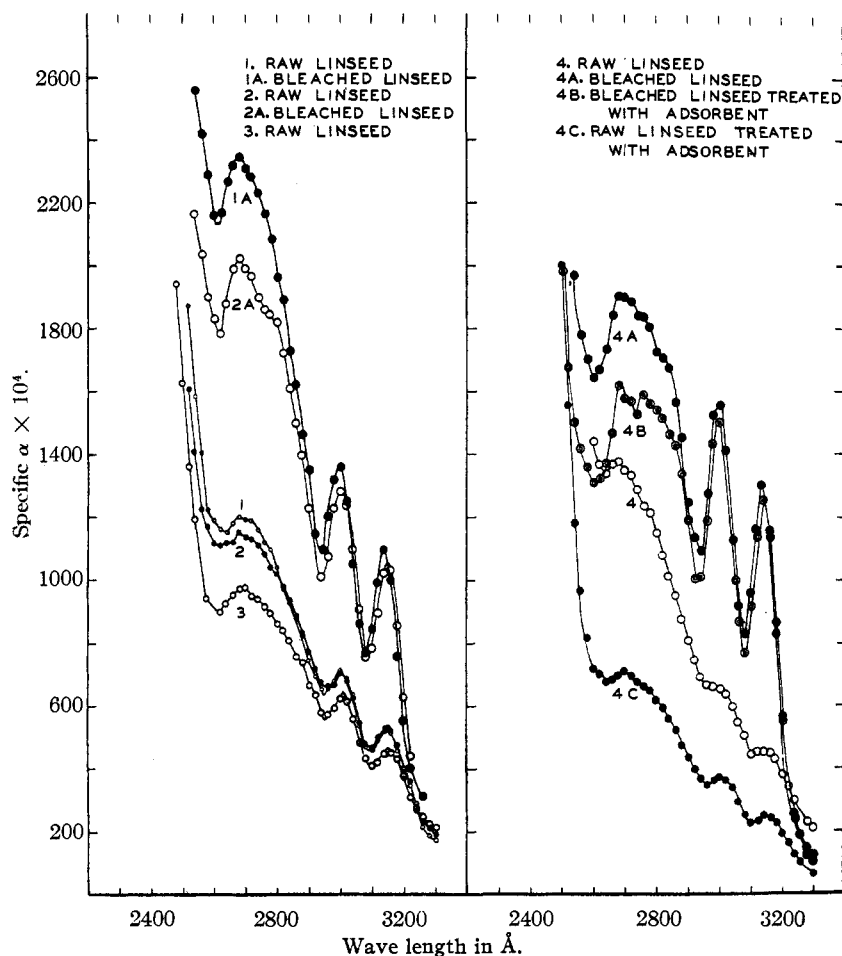


Fig. 1.—Absorption spectra of linseed oils.

The curve for methyl linoleate is higher after bleaching than before. It does not show the bands at  $3000$  and  $3160 \text{ \AA}$ . which were present in linolenic ester. The shape of the curve for ethyl linoleate was the same as that of methyl linoleate. The curves for linoleic esters are like that of bleached corn oil, 3, which contains a high percentage of linoleic acid but in which no linolenic is found by chemical methods. They are also like the curve of unbleached soybean oil to which about 0.10% of tung oil has been added, curve 2. In tung oil this band is due to the presence of eleostearic ( $\Delta^9,10,11,12,13,14$ -octadecatrienoic) acid.<sup>9</sup> There is no evidence for diene conjugation in the linoleic ester, since there is no band at  $2325 \text{ \AA}$ .

**The Effect of Repeated Bleaching on the Absorption Spectra of the Oils.**—It was impossible to increase the height of the absorption maxima by repeating the bleaching process or by increasing the amount of earth. Thus, it was apparent that there is a limiting factor in the formation of the substances causing these bands.

**Oxidation of the Oil before Bleaching.**—Oxidation of the oil with air before bleaching was found to be necessary for the development of absorption bands during the fuller's earth treatment. Alkali refined soybean oil was blown with air for nine hours at  $100^{\circ}$ . This oil gave absorption

(9) Dingwall and Thomson, *THIS JOURNAL*, **56**, 899 (1934).

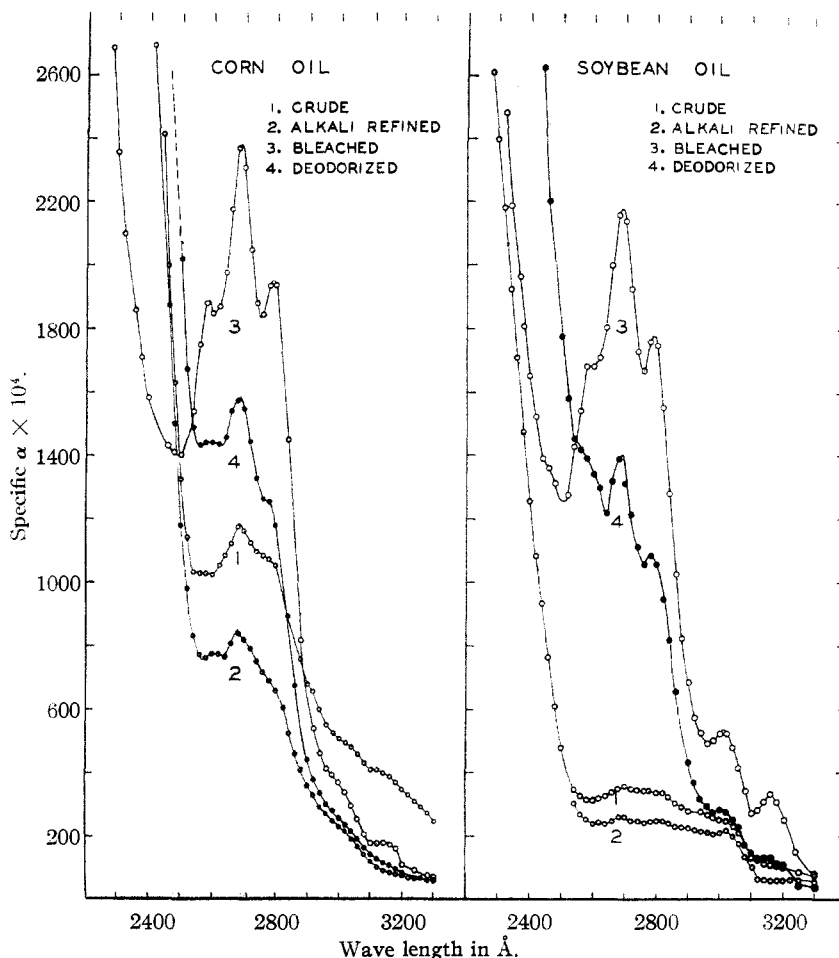


Fig. 2.—Absorption spectra of processed corn and soybean oils.

curve 1, Fig. 4, while after it was bleached with fuller's earth, curve 2 was obtained.

Alkali refined cottonseed oil, perhaps due to a greater stability toward oxidation, showed hardly any increase in absorption after bleaching, curve 1, Fig. 4; however, after this oil was oxidized by blowing with air and then bleached with the same earth, curve 2 was obtained.

With all of the oils, the bands were found to disappear upon oxidation with air and to reappear much more intensely when the oil was bleached.

**Spectra of Linseed Oil Fatty Acids Obtained by Low Temperature Crystallization.**—The mixed fatty acids of bleached linseed oil were fractionally crystallized<sup>10</sup> from acetone. The most unsaturated fraction was recrystallized four times from petroleum ether (65.5–66.5°) at –60°. While no concentration of the absorbing material was obtained, the substance responsible for the absorption at 2680 Å. was removed, revealing the presence of another band at 2860 Å., Fig. 5. This spectrum is compared with that of the four double bond conjugated parinaric ( $\Delta^9:10,11:12,13:14,15:16$ -octadecatetraenoic) acid which was obtained by Tsujimoto<sup>11</sup> from the seed fat of *Parinarium*

*laurinum*. The spectrum of this acid was obtained by Kaufmann<sup>12</sup> who compared it with that of conjugated decatetraene prepared by Kuhn.<sup>13</sup>

Numerous possibilities exist for *cis-trans* isomerism in compounds of this type. In other compounds such as stilbene<sup>14</sup> and the  $\alpha$ - and  $\beta$ -eleostearic acids,<sup>9</sup> such isomerism has been shown to shift the position of the absorption maxima somewhat. This may be the explanation for the fact that the maxima in parinaric acid and decatetraene do not coincide.

### Discussion

Hulst<sup>15</sup> in a study of the ultraviolet absorption spectra of the fatty acids noted the presence of three maxima at 270, 302, and 315  $m\mu$  in ethyl linolenate. He also found general absorption in the region of 270  $m\mu$  for ethyl linoleate, but no detail is apparent in his absorption curves. He stated that the most pure samples showed least absorption.

Bradley and Richardson<sup>16</sup> noted the presence of two weak absorption bands at about 3000 and 3160 Å. in the spectra of linseed and soybean esters but gave no interpretation of the phenomenon.

Brode and Tryon<sup>17</sup> reported that bands which they observed in linoleic and linoleic acids are due to the fact that in the chain of atoms some unsaturated linkages are further from the carboxyl group than others, and that even in non-conjugated acids one should expect to find, with an increasing number of unsaturated linkages, an increasing number of band maxima. This view, however, with regard to the bands at wave lengths longer than 2200 Å., is not supported by the present study.

(12) Kaufmann, Baltes and Funke, *Fette und Seifen*, **45**, 302 (1938).

(13) Kuhn and Grundmann, *Ber.*, **71B**, 442 (1938).

(14) Smakula and Wassermann, *Z. physik. Chem.*, **A155**, 353 (1931).

(15) Hulst, *Rec. trav. chim.*, **54**, 639 (1935).

(16) Bradley and Richardson, *Ind. Eng. Chem.*, **32**, 963 (1940).

(17) Brode, "Chemical Spectroscopy," John Wiley and Sons, Inc., New York, N. Y., 1939, p. 136.

(10) Shinowara and Brown, *THIS JOURNAL*, **60**, 2734 (1938).

(11) Tsujimoto and Kayanegi, *J. Soc. Chem. Ind., Japan*, **36**, 110B, 673B (1933).

The isolated double bond between carbon atoms has been found to produce an absorption band with a maximum at 1800 Å. The

methylene group,  $\text{H}-\overset{\text{H}}{\underset{|}{\text{C}}}-\text{H}$ ,

when it occurs between double bonds has an insulating effect and when it occurs between two parts of a conjugating system, may be likened<sup>18</sup> to an insulator in an electrically conducting system, dividing it into two parts. Thus, from theoretical considerations it is not possible to regard any absorption bands in the region of the spectrum of wave length longer than 2200 Å. as being characteristic of non-conjugated linoleic and linolenic acids.

Conjugation, however, is of great importance in causing absorption in this region as is illustrated by the specific absorption of 170 at 2700 Å. found by Dingwall and Thomson<sup>9</sup> for  $\alpha$ -eleostearic acid and of 120 at 2300 Å. for 9,11-linolenic acid found by Hulst.<sup>15</sup>

It has been found by certain investigators<sup>19,20</sup> that refluxing with alkali brings about conjugation of the double bonds in  $\Delta^{9,12}$ -linoleic and  $\Delta^{9,12,15}$ -linolenic acids. This, however, is not the change which occurs during bleaching. Treatment of oxidized linoleic esters with bleaching earth produces a band at 2680 Å. characteristic of triene conjugation. Alkali treatment gives a band at 2325 Å. due to diene conjugation. Treatment of oxidized linolenic esters with bleaching earth produces bands at 2860, 3000 and 3160 Å. characteristic of tetraene conjugation, as well as a band at 2680 Å. Alkali treatment of linolenic esters gives a band, due to triene conjugation, at 2680 Å. and a band, due to diene conjugation,

(18) Lewis and Calvin, *Chem. Rev.*, **25**, 273 (1939).

(19) Moore, *Biochem. J.*, **31**, 138 (1937).

(20) Kass and Burr, *THIS JOURNAL*, **61**, 3292 (1939).

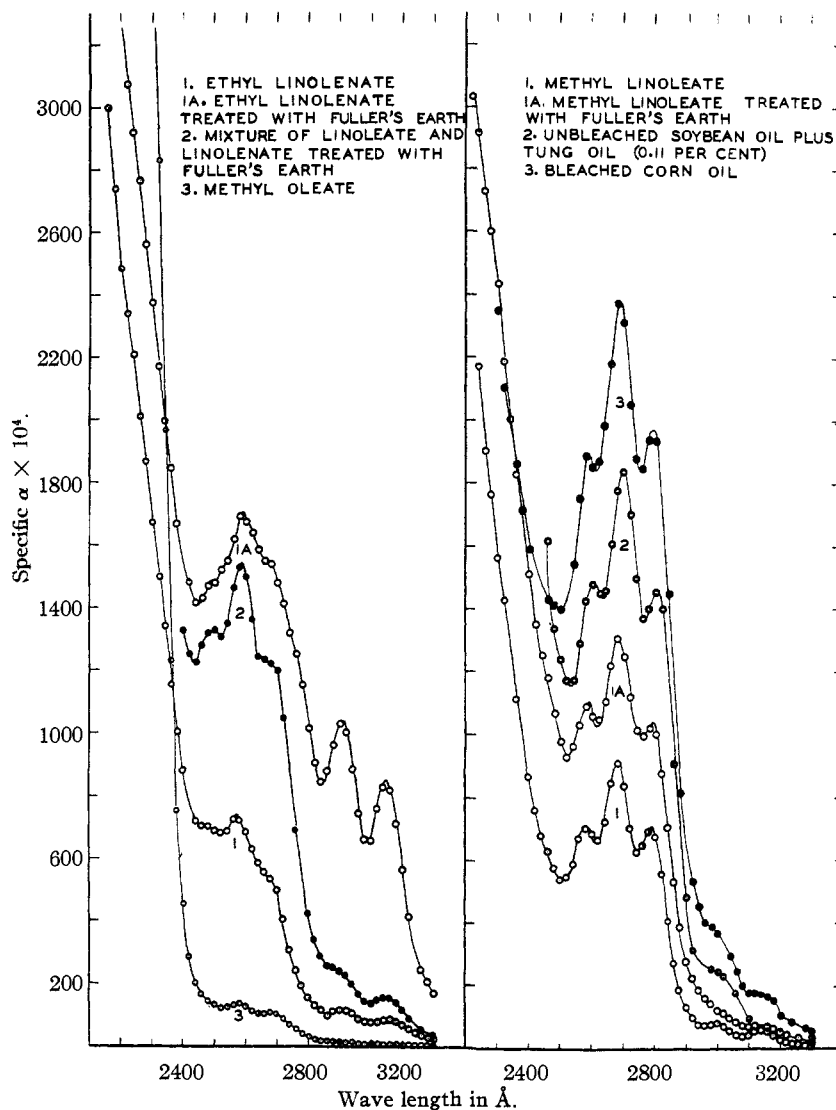


Fig. 3.—Comparison of absorption spectra of "bleached" linoleic and linolenic esters with those of bleached oils.

at 2325 Å. Thus, alkali treatment results in a shift of the double bonds, already present, to conjugated positions but bleaching with fuller's earth appears to cause the introduction of a new double bond in linoleic and linolenic acids and in such a way that they are conjugated. The amounts of conjugated material produced in the latter case are very small.

Study of our experimental evidence indicates that from linoleic ester a small quantity of a triene conjugated system is formed while from linolenic ester small quantities of both triene and tetraene conjugated systems are formed. Oxidation of the oil and fatty acid esters, before treatment with fuller's earth, is necessary for the formation of

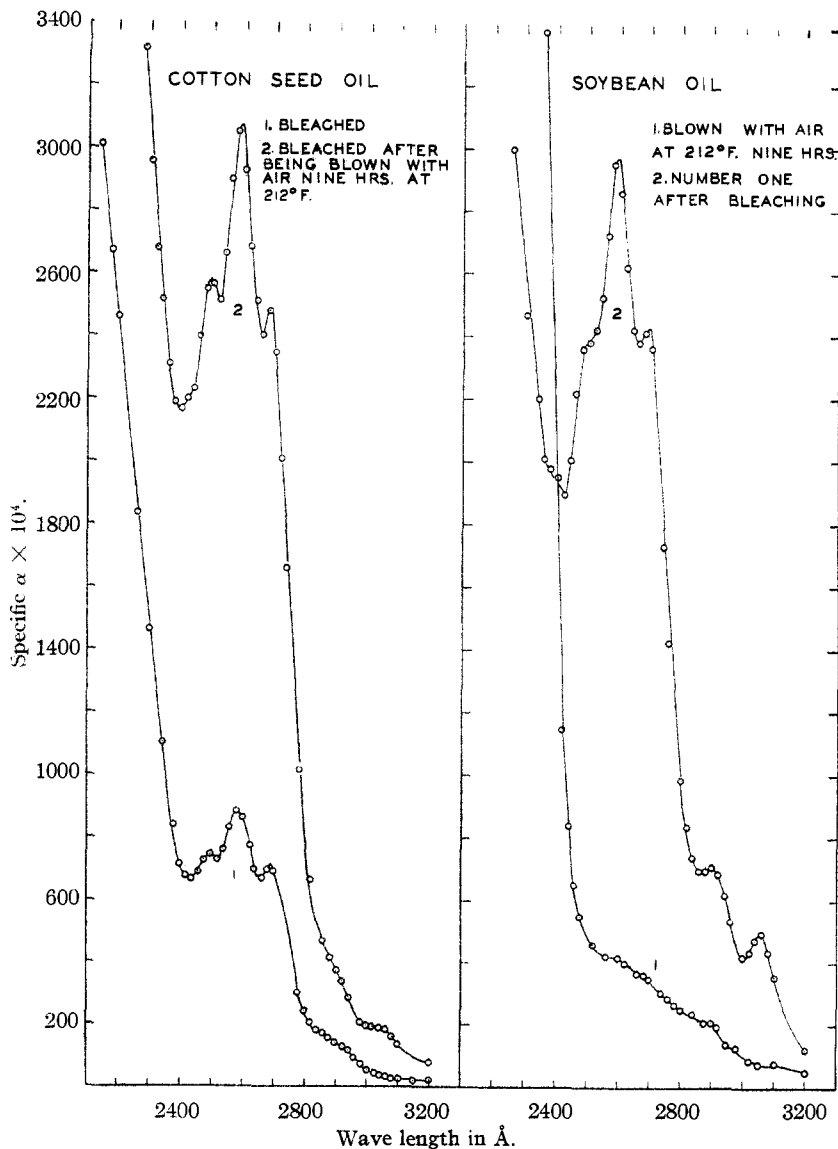


Fig. 4.—Absorption spectra of oils bleached after oxidation by air.

*vacuo*. This occurs during the first stages of treatment, as would be expected, since conjugated aliphatic acids are easily oxidized and polymerized.

The lower absorption found for deodorized oils, Fig. 2, appears to be due to polymerization of a part of the conjugated compounds as a result of the high temperatures used in this process. In some instances<sup>22</sup> such temperatures may range as high as 274°.

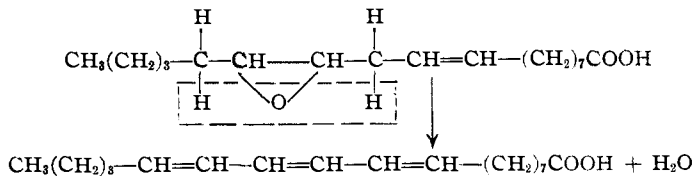
**Possible Mechanisms of Formation of the Conjugated Systems.**—Assuming the formation of an ethylene oxide ring during oxidation of fats, as has been proposed,<sup>23</sup> dehydration might take place as is illustrated with linoleic acid to give a conjugated system containing another double bond. The hydrogen atoms attached to the number eleven carbon atom are activated by the double bond, and this condition would be expected to aid in the dehydration.

Another possibility is dehydration of a hydroxy compound which might be

formed as a result of oxidation of the active earth.

It is believed that the earth, perhaps due to the presence of a trace of acid, acts as a dehydrating agent on an oxidized product of linoleic and linolenic acids to cause the introduction of a new double bond in such a way that small quantities of conjugated compounds are formed.

In a previous investigation<sup>21</sup> it was reported that the bands present in linseed oil disappear when the oil is bodied by blowing with air at elevated temperatures or bodied by heating in



methylene group between the double bonds. Scheiber<sup>24</sup> believes that this group is attached by oxygen to form keto compounds.

Dehydration initiated by removal of a hydroxyl group on the carbon atom between the

(21) Mitchell and Kraybill, *Ind. Eng. Chem., Anal. Ed.*, **13**, 785 (1941).

(22) Bailey, *Ind. Eng. Chem.*, **33**, 404 (1941).

(23) Deatherage and Mattill, *ibid.*, **31**, 1425 (1939).

(24) Scheiber, *Z. angew. Chem.*, **46**, 643 (1933).

double bonds could result in a shift of electrons and the expulsion of a proton to give a triene conjugated system. In this reaction the carbon atoms left with only six electrons are indicated with a star.

If by oxidation of linolenic acid an ethylene oxide ring is formed between carbon atoms 12 and 13, dehydration could give a tetraene conjugated system. Dehydration occurring with an oxide ring on either of the other two double bonds would give a three double bond system with the fourth double bond in an isolated position where it would not affect the absorption spectrum. That is, the spectrum of such a compound would be expected to have a maximum in the 2700 Å. region, characteristic of triene conjugation, and another below 2200 Å. due to the single double bond.

If a hydroxyl group, formed by oxidation, were removed from a carbon atom between two double bonds both a triene and a tetraene bond conjugated system could be formed, depending upon the direction of the electronic shift.

Further study along this line may disclose the nature of certain of the oxidation products of some fats as well as of other organic compounds containing similar groupings of double bonds.

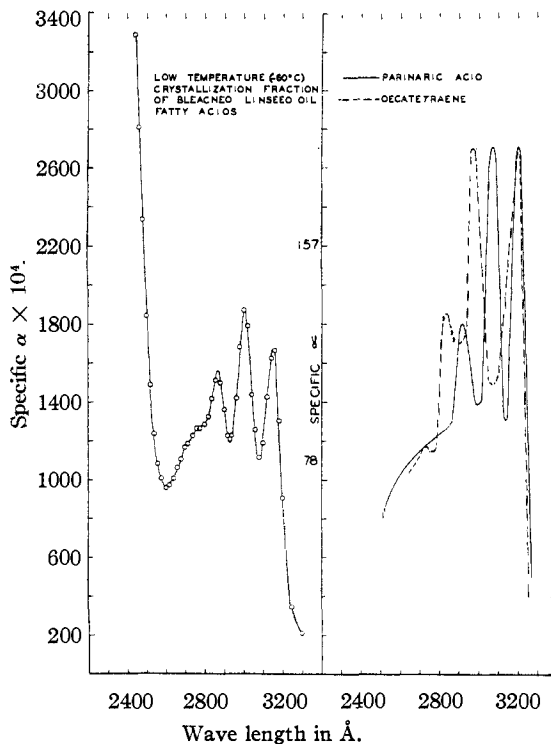
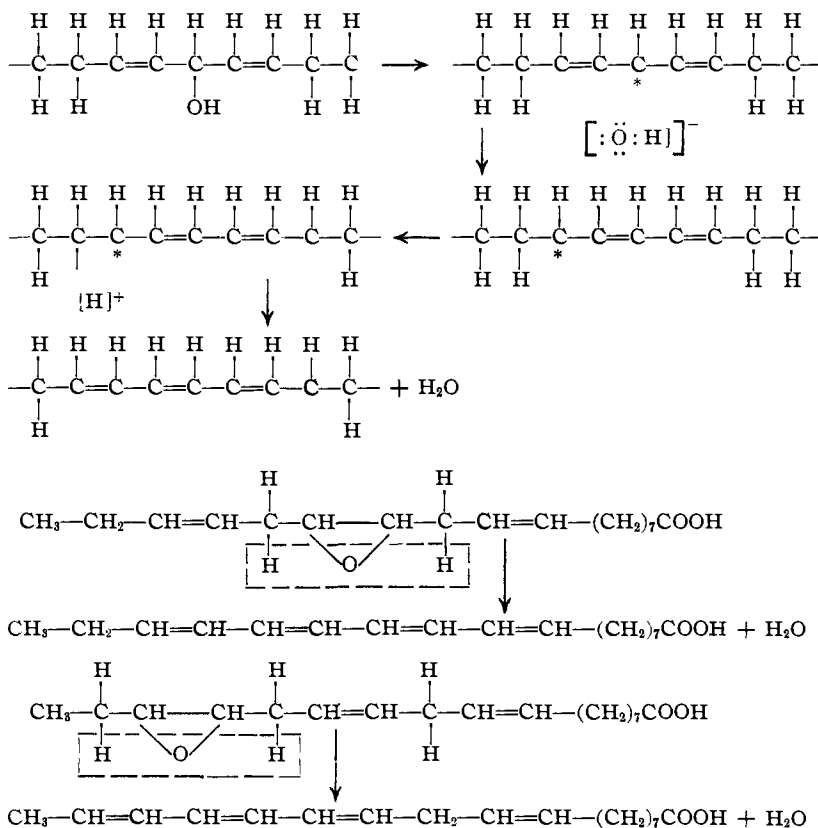
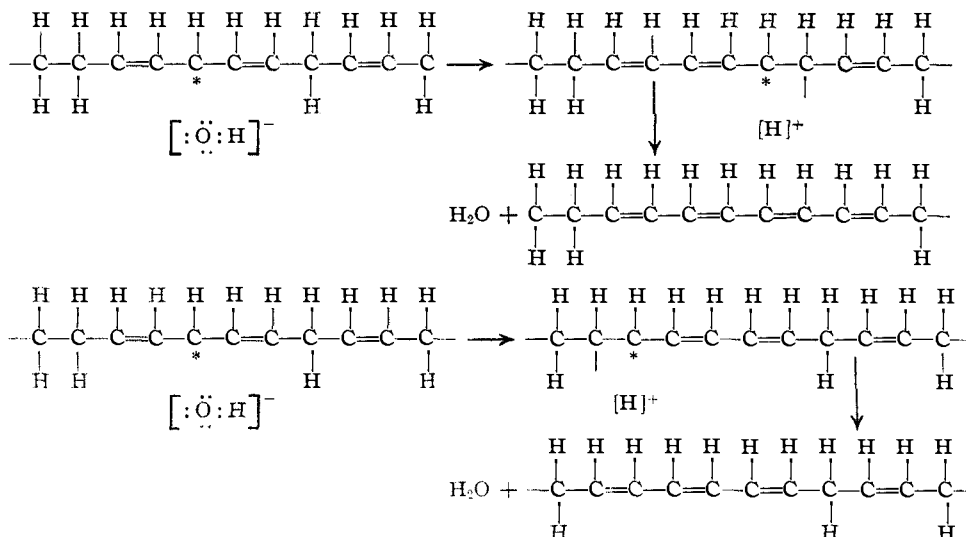


Fig. 5.—Comparison of absorption spectra of tetraene conjugated systems with that of -60° fraction of bleached linseed oil fatty acids.

### Summary

1. Alkali refining of vegetable oils as well as refining by adsorption with a special silicate adsorbent results in a decrease of absorption in the ultraviolet region. This is thought to be due to removal of some of the non-fat constituents.

2. Bleaching with fuller's earth causes the development of definite absorption bands. Oils containing only linoleic acid develop bands at 2680 Å. while oils containing both linoleic and linolenic acids develop bands at 2680, 3000 and 3160 Å. The band at 2680 Å. is due to a triene conjugated system which is formed from linoleic and linolenic acids, while the bands at 3000 and 3160 Å. are due to a tetraene conjugated system formed from linolenic acid.



3. By low temperature crystallization from solvents, the most unsaturated fraction of bleached linseed oil fatty acids was obtained. This process removed the substance causing a band at 2680 Å., leaving the highly unsaturated fraction with a spectrum similar in character to that obtained for the four double bond conjugated system in decatetraene and in parinaric acid.

4. Oxidation of the oil or fatty acid esters is a necessary intermediate step in the formation of the conjugated systems which may be produced by dehydration.

5. Pure non-conjugated linoleic and linolenic acids would show no absorption bands in the region of wave length longer than 2200 Å. Any

bands found in regions of longer wave length are due to the presence of traces of conjugated materials.

6. There was no evidence for diene conjugation in linoleic and linolenic acids prepared by debromination.

7. In the bleached oils examined, the amounts of conjugated material were very small, about 0.1 or 0.2%.

8. Deodorization results in a lowering of these bands due to polymerization of the conjugated systems at elevated temperatures. The bands are removed in the processes of bodying linseed oil by heating *in vacuo* or by oxidation.

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RECEIVED JANUARY 2, 1942

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## Kinetics of Gaseous Reactions by Means of the Mass Spectrometer. The Thermal Decomposition of Dimethyl Ether and Acetaldehyde\*

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The kinetics of gaseous reactions have been the subject of a great many investigations not only because of their intrinsic interest, but also with the hope of securing a clearer insight into the mechanisms of the reactions. These studies have been made principally by two methods, namely, by the measurement of pressure and by detailed analyses of the reaction mixture by chemical and physical means. Most of the data reported in

\* Presented at the Atlantic City Meeting of the American Chemical Society.

the literature for kinetics of gaseous reactions have been found by pressure measurements because of the ease with which they may be made. If one then assumes an appropriate mechanism consistent with the data, a rate constant may be calculated. The obvious disadvantage of the pressure method is that it gives no insight into the nature of the reactions, which actually take place; that is, any intermediates, which are formed, may not be detected. Because of this, the tendency in recent work in gas reactions has