dissolved or so thoroughly emulsified in the water as to make complete recovery difficult if not impossible.

Distillation losses have been variously reported as being from a few tenths of 1% to as much as 2%. It is probable that the combined losses are somewhere between 0.5% and 1% for continuous distillation. Distillation losses can be held to a minimum by:

1. Distilling at the highest vacuum and lowest steam ratio practicable.
2. Distilling by a continuous process in corrosion resistant equipment rather than by a batch process in iron equipment, such as the old type pot stills. Decomposition is greater by batch distillation, particularly in iron stills.
3. Providing adequate condenser capacity so as to maintain carry-over at a minimum. Reduction of carry-over with the steam by installation of baffles or bubble caps in the vapor line between the condenser and barometric has long been known.
4. Splitting of residues so as to keep the neutral fat content of the feed to the tar stills as low as possible.

Spent bleaching clay resulting from the bleaching of stearic acid contains from 25% to 35% of stearic acid. On the basis of using 3% of clay to bleach the stearic acid, this represents a loss of about 1% of the stearic acid bleached or 0.4% of the raw fat. Practically all of the stearic acid can be recovered from the spent clay by solvent extraction.

The need for adequate catch basins in a fatty acid plant cannot be over-emphasized. Much of the fat and fatty acids drawn off with water and emulsions from processing tanks will separate in the catch basins and can thus be reclaimed. The sewer system leading to the catch basins should preferably carry only processing liquors so as to keep the amount of water and therefore the flow through the catch basins to a minimum. Separate pumps for each department are also desirable in order to eliminate degrading of stocks as much as possible.

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Applied Ultraviolet Spectrophotometry of Fats and Oils

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RECENT developments in spectrophotometric methods and equipment have brought about greatly increased use of ultraviolet spectrophotometry as applied to many chemical problems. Studies of fats and oils are no exception. Within the past few years a number of reference materials have been prepared and purified and used as spectrophotometric reference standards. Studies regarding the empirical isomerization of double-bond systems to bring about conjugated systems have enabled the development of quantitative spectrophotometric methods for analysis of fats and oils for their polyunsaturated constituents (1, 2, 3, 4, 5, 6, 7, 8). These methods are so simple and rapid as compared with earlier chemical procedures, and their sensitivity is so great, that there is an increasing use of spectrophotometric studies on a routine laboratory basis. The recent application of this type of method to the control of tallows and soaps is an outstanding example of such use (7, 8). Because of the rapid growth of developments in the field it is considered advisable at this time to discuss the methods of spectrophotometric analysis of fats and oils and to point out some of their applications in industry. The discussion will include sample calculations, interpretation of the absorption spectra, and other similar considerations. The studies to be discussed depend upon the fact that there are certain structures in the fat molecules known as chromophores, which absorb radiant energy in a characteristic manner. These chromophores with which we are concerned in the fatty acids are composed of double bonds. Figure 1 shows a simplified diagram of the double bond systems in several of the naturally occurring fats or fatty acids. Note that in linoleic acid and linolenic acid, as well as in arachidonic acid, the double bonds are separated from each other by two single bonds. This structure leaves the double bonds in more or less isolated systems, and they do not absorb radiant energy characteristically in the ultraviolet portion of the spectrum at wavelengths above 2100 Å. Although these systems do show characteristic absorption at shorter wavelengths, i.e. in the "vacuum ultraviolet" (9), the present discussion will be confined to the region which may be studied by an instrument such as the Beckman Model DU quartz spectrophotometer (10).

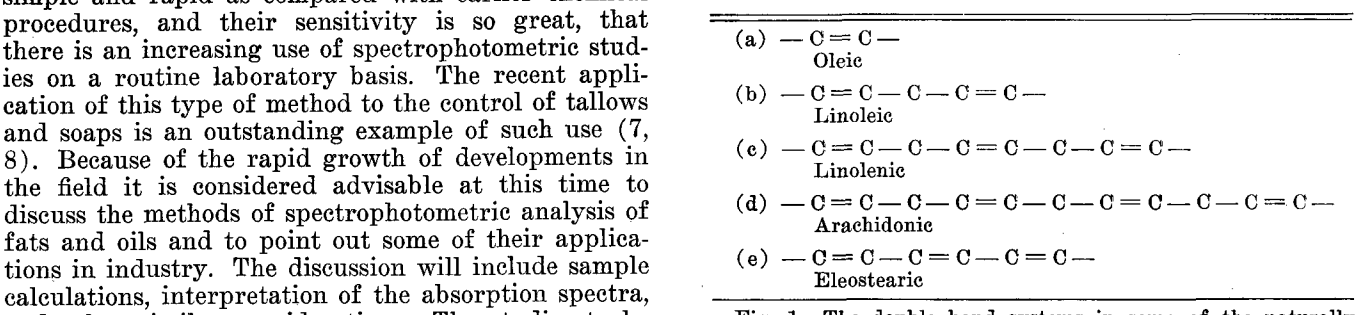

Fig. 1. The double bond systems in some of the naturally occurring fatty acids.

Because the double bonds are in these isolated positions, the ultraviolet absorption spectra of oils containing the double bond systems shown in (a), (b), (c), and (d), of Figure 1 do not possess characteristic shapes. When two or more of these double bonds are

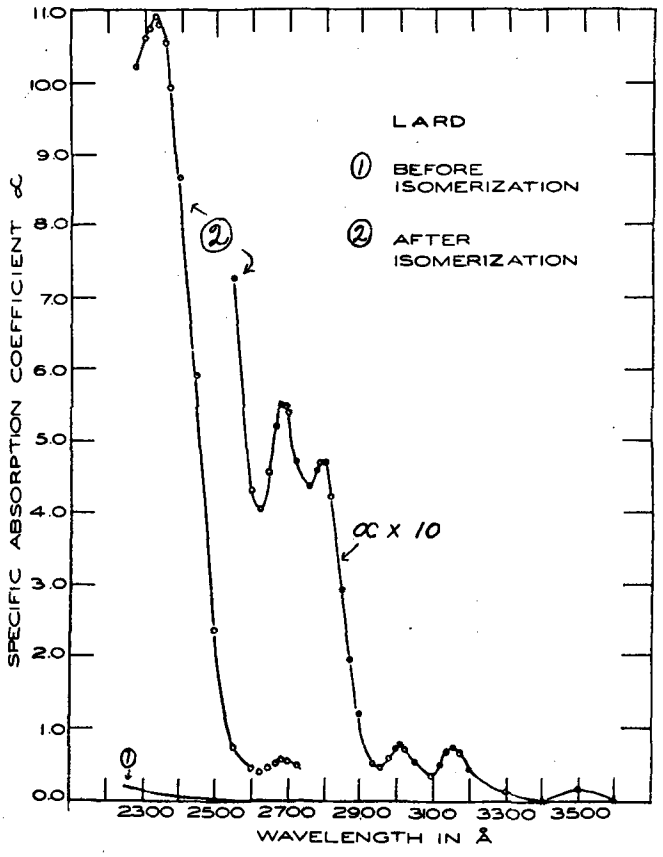

FIG. 2. Ultraviolet absorption spectrum of lard as observed before and after the alkali isomerization treatment of Mitchell *et al.* (5).

in conjugated position, as shown in line (e) of Figure 1, these double bond systems absorb energy in characteristic fashion, and their absorption spectra possess definite bands or maxima. For example, two double bonds in conjugation as in 9,11-octadecadienoic acid, produce an absorption maximum at about 2340 Å. Three double bonds in conjugation, as in eleostearic acid, produce maximum absorption at about 2680 Å, while four double bonds in conjugation, as in parinaric acid, produce maximum absorption at about 3010 and 3160 Å. It is evident therefore that, by examining a fat or oil in a suitable solvent at the regions of the wavelengths mentioned, it is possible to learn whether or not these conjugated systems exist.

It is readily ascertained by the examination of the ultraviolet absorption spectra that most of the more common naturally occurring fats and oils of vegetable and animal origin do not contain very large amounts of constituents with conjugated double bonds. The evidence for this conclusion is the lack of characteristic absorption maxima in the absorption curves. Tung oil and the oil from *Parinarum Laurinum* are notable exceptions. Brice *et al.* (8) have reported considerable amounts of conjugated material in tall oil and perilla oil.

It has been known for some time that the position of double bonds in a hydrocarbon chain may be shifted by exposure to alkali and heat (1, 2, 3, 5). Moore observed, for instance (1), that when saponified fats are subjected to prolonged heating, the non-conjugated double bonds become conjugated and absorb radiation in the ultraviolet region above 2100 Å. Other workers, notably Kass *et al.* (2) and Miller and Burr (3) found that it was possible to measure quantitatively the amount of linoleic acid in vegetable oils by spectrophotometric studies after such an alkali treatment. Mitchell, Kraybill, and Zscheile (5) studied the conditions for alkali isomerization, devised spectrophotometric reference standards for purified linolenic and linoleic acids, and proposed a method for the quantitative analysis of fats for the three-double-bond and two-double-bond unsaturated acids and, making use of the iodine value of the fat, the monoethenoid and saturated acids. Thus a relatively complete fat analysis was made possible on the basis of spectrophotometric data and iodine value. This method assumes that the unsaturated constituents consist of oleic, linoleic, and linolenic acids. Beadle and Kraybill (6) later published reference values for arachidonic acid, as well as for linoleic and linolenic acids, as obtained with the Beckman spectrophotometer. The spectrophotometric method was thus extended to include the tetraethenoid acids calculated as arachidonic.

As an example of the use of this method consider Fig. 2, which shows the absorption spectra of lard before and after alkali isomerization has taken place (11). Note that in the curve which represents the lard

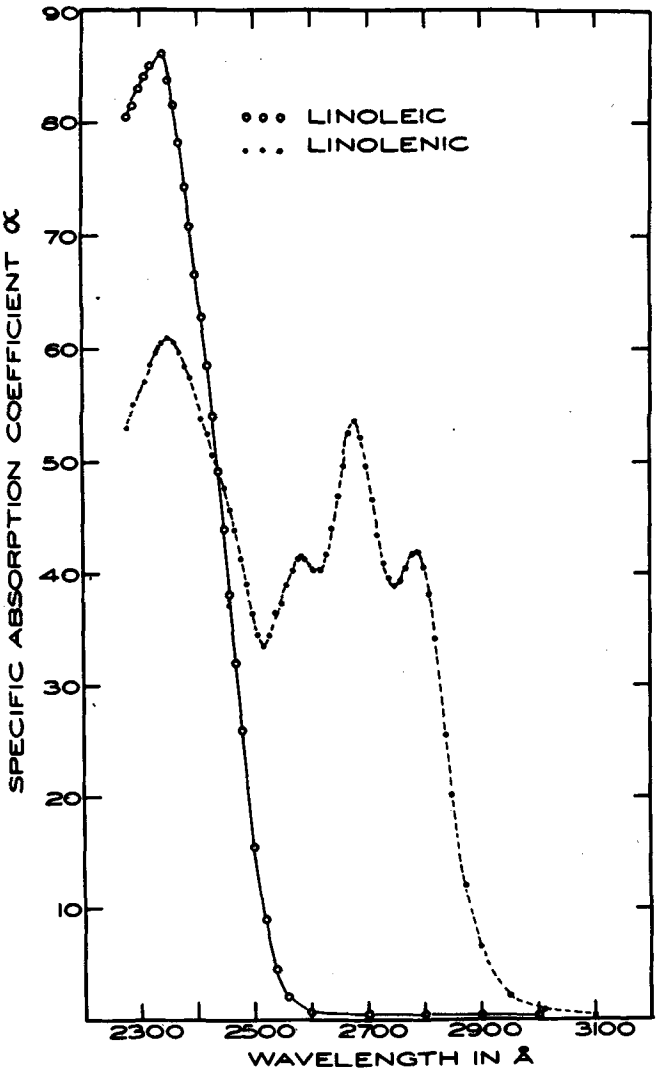

FIG. 3. Ultraviolet absorption spectra of the alkali isomerized soaps of purified linoleic and linolenic acids in ethanol (6).

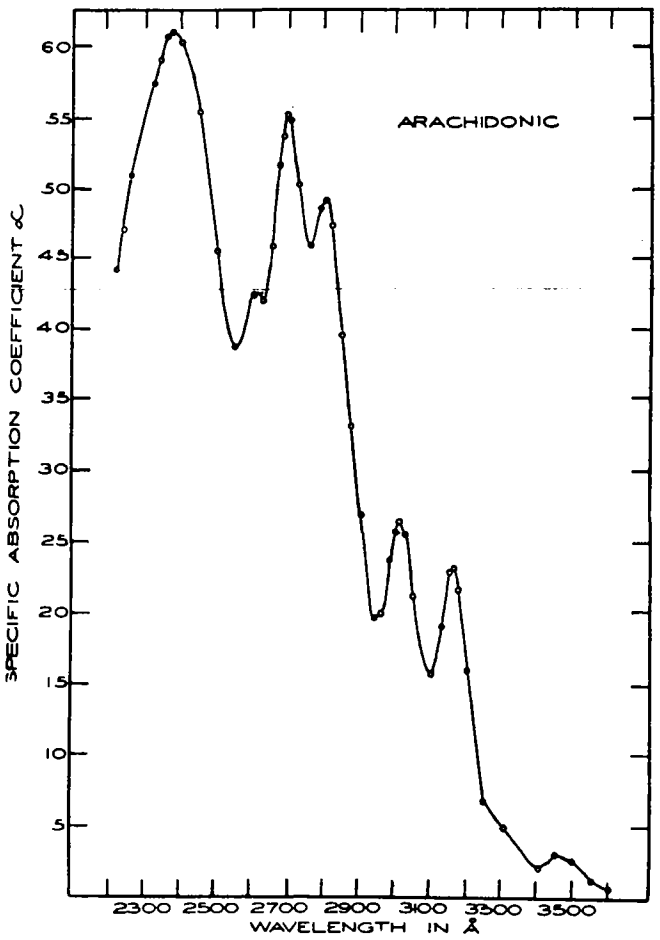

FIG. 4. Ultraviolet absorption spectrum of the alkali isomerized soaps of arachidonic acid in ethanol (6).

before treatment there is a virtual absence of any characteristic structure although there is a general slow rise at the shorter wavelengths. The absorption spectrum of the same lard after being subjected to the alkali isomerization should be compared with Figures 3 and 4, which show the spectra of the alkali

TABLE 1

Reference Values for Use in Spectrophotometric Analysis of Fats

Isomerized Fatty Acid	Specific Absorption Coefficients*		
	2340 Å	2680 Å	3160 Å
Arachidonic	59.3	53.4	22.6
Linolenic	60.9	53.2	.....
Linoleic	86.0	.....	.....

* From data on the isomerized soaps in ethanol. Values obtained using the Beckman Model DU Quartz spectrophotometer.

isomerized soaps of linoleic, linolenic, and arachidonic acids. Observe that the maxima shown on the lard curve at 3010 and 3160 Å correspond to those for arachidonic acid at those wavelengths. Note further the similarity of the maxima near 2680 Å to the corresponding maxima for isomerized linolenic acid, and the similarity of the maximum at 2340 Å to that for isomerized linoleic acid. By referring to the reference values for these acids as shown in Table 1, it is possible to calculate the four-double-bond, the three-double-bond, and the two-double-bond acids on the assumption that they are arachidonic, linolenic, and linoleic, respectively. Then, by making use of the iodine number of the lard, it is possible to calculate the monoethenoid acid content. These calculations are based on the use of the Lambert-Beer equation, which may be stated as

$$\log \frac{I_o}{I} = acl, \text{ or } a = \frac{\log \frac{I_o}{I}}{cl}$$

in which

I_o = the intensity of radiation transmitted by the solvent (or "blank"),

I = the intensity of radiation transmitted by the solution,

a = specific absorption coefficient,

c = concentration of solute in grams per liter,

and l = length of optical path through solution, in centimeters.

The value $\log \frac{I_o}{I}$ is obtained as the "D" reading on the Beckman instrument, or in any other suitable manner. Since the c and l values are known, it is a simple matter to solve for a. After sufficient readings have been made to establish that maxima are present, the a values are calculated from the "D" readings at 3160, 2680, and 2340 Å. (Usually the reading of "D" values at 3200, 3160, 3100, 2700, 2680, 2660, 2340, and 2300 Å will establish the presence or absence of maxima.) Because the analysis is based on the fatty acids present, all the a values are multiplied by the factor 1.046 to convert the values from the glyceride basis to the mixed acids basis.* The iodine number also is multiplied by this factor. Below are the equations for the quantitative analysis of the lard whose spectrum is shown in Figure 2:

a 3160 Å $= 0.0712 \times 1.046 = 0.0745 = a_1$
a 2680 Å $= 0.550 \times 1.046 = 0.575 = a_2$
a 2340 Å $= 10.8 \times 1.046 = 11.30 = a_3$
I_2 value $= 67.3 \times 1.046 = 70.4$

Arachidonic Acid =

$$\frac{a_1}{22.6} \times 100 = \frac{0.0745}{22.6} \times 100 = 0.33\% = W$$

Linoleic Acid =

$$\frac{a_2 - \left(\frac{W}{100} \times 53.4\right)}{53.2} \times 100 =$$

$$\frac{0.575 - (0.0033 \times 53.4)}{53.2} \times 100 = 0.75\% = X$$

Linolenic Acid =

$$\frac{a_3 - \left(\frac{W}{100} \times 59.3\right) - \left(\frac{X}{100} \times 60.9\right)}{86.0} \times 100 =$$

$$\frac{11.30 - (0.0033 \times 59.3) - (0.0075 \times 60.9)}{86.0} \times 100 = 12.4\% = Y$$

Oleic Acid =

$$\frac{(I_2 \times 100) - (W \times 333.5\dagger) - (X \times 273.8\dagger) - (Y \times 181.5\dagger)}{90\dagger} =$$

$$\frac{7040 - (0.33 \times 333.5) - (0.75 \times 273.8) - (12.4 \times 181.5)}{90} =$$

$$49.7\% = Z$$

* Work done by Dr. J. H. Mitchell, Jr. of our laboratories indicates that the fatty acids in a number of fats studied comprise about 95.6% of the fat molecule.

Saturated Acids =
$100 - W - X - Y - Z =$
$100 - 0.33 - 0.75 - 12.4 - 49.7 = 36.82\%$

† Iodine values for the pure acids.

It may be observed that the above calculations do not correct for background absorption, except by virtue of the blank. It is extremely important that all spectrophotometric readings be made using the blank in the solvent cell and that the blank be diluted in the same manner as are the samples. It is assumed in these calculations that the linoleic acid does not absorb at 2680 Å and that neither the linoleic nor the linolenic absorb at 3160 Å. It may be observed from Figure 3 that interference of this type is slight.

Applications

Table 2 shows a comparison of data obtained on the esters of human milk fat by the spectrophotometric

TABLE 2

Analysis of Esters from Human Milk Fat

	Beadle and Kraybill	Brown and Orians	
	Spectrophotometric	S C N	Ester Fract.
Arachidonic	1.0	1.0	0.7
Linolenic	0.8	....	....
Linoleic	12.2	12.3	11.0
Monoethylenic	41.4	42.5	42.8
Saturated	44.9	44.2	45.5

method and by other methods of analysis, including the thiocyanogen reaction and ester fractionation. The spectrophotometric analyses were made in our laboratory and the other analyses were made in the laboratories of J. B. Brown at Ohio State University (16). As shown by the table, agreement among the methods is good, with the exception that the spectrophotometric method indicated the presence of a small amount of linolenic acid while the other methods did not.

In the recently proposed modified method by Brice *et al.* (7, 8) equations are set up which have as one of their aims the correction for background absorption. The principal objective of the method is to provide a spectrophotometric means of detecting very small amounts of polyunsaturated acids, both conjugated and non-conjugated. This is the method which has been applied to quality control for tallows and soaps as outlined in a Rubber Reserve Company Manual (12). By its use, analysis of curves with very slight absorption maxima is facilitated so that polyunsaturated constituents occurring in very small amounts may be calculated.

In addition to the general analytical problems in which spectrophotometry has been applied to fats and oils, the method has application in those instances where variations in the double bond systems are found with difficulty or not at all by the ordinary chemical methods. The control of tallows and soaps mentioned above is one example. Another application is found in the study of corn oil during its processing.

Work in our laboratories had shown that a small amount of conjugated material was present in samples of refined corn oil which we had investigated. We were unable to find comparable amounts in the crude oil. Accordingly, samples of the oil were obtained at various stages of processing, with the results

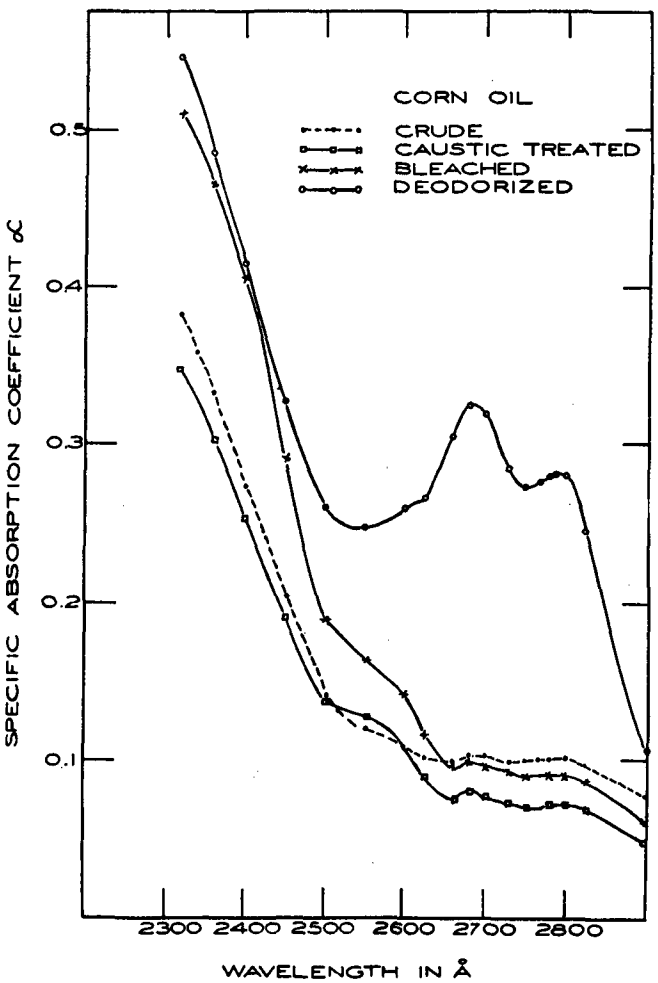

FIG. 5. Ultraviolet absorption spectra of corn oil following various stages of processing.

shown in Figure 5. Note that the curve for the refined deodorized oil is the only one which shows prominent absorption maxima in the 2680 Å region, showing quite clearly that most of the triene conjugation which was present was formed during this step in processing. This conjugated material may have arisen either from triene materials already present or from a small proportion of the diene materials, as suggested by Mitchell and Kraybill (13). Formation of this conjugated system might be of considerable interest to those who are studying flavor reversion and stability problems. The total amount of conjugated triene acid present is small, and a study of its occurrence would be extremely difficult by any method in use at the present time other than spectrophotometric. Obviously, studies of the type illustrated by Figure 5 may be applied to the processing of any fat or oil.

Another very interesting application of the use of spectrophotometry is in connection with studies being made to improve the drying properties of oils such as linseed. As is known, tung oil contains three-double-bond conjugated materials which are believed to contribute greatly to its high quality as a drying oil. Linseed oil contains three-double-bond acids in its glycerides, but the double bonds are not conjugated. Considerable attention has been devoted to studies of catalytic means of shifting the double bonds in linseed oil to form the conjugated system of the eleostearic acid in tung oil. Because the non-

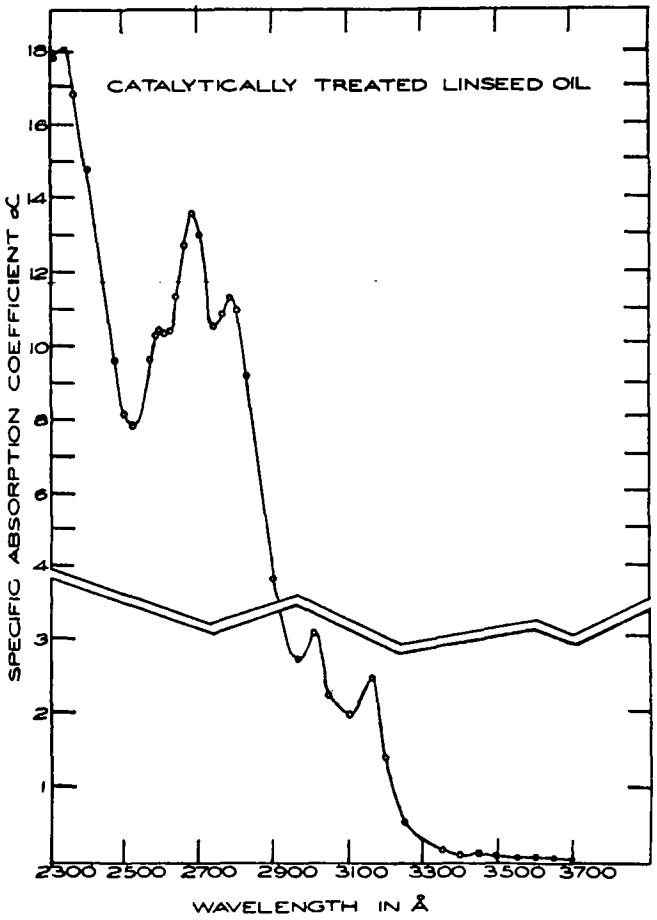

FIG. 6. Ultraviolet absorption spectrum of linseed oil following catalytic treatment during drying oil studies.

conjugated linseed oil does not yield an absorption spectrum with the maxima characteristic of eleostearic acid, the progress of catalytic conjugation may be followed by the appearance of these maxima. The intensity of the absorption is a measure of the amounts formed. Figure 6 shows the absorption spectrum of a sample of linseed oil which had been catalytically treated in an attempt to improve its drying properties. It is important to emphasize that this sample was not subjected to alkali isomerization but was merely dissolved in a suitable solvent for examination. Note the well-defined absorption maxima at 3010 and 3160 Å, as well as at 2680 Å and 2340 Å. These maxima are excellent evidence that considerable conjugation was produced; and, furthermore, that this conjugation was not only of the triene type but was also diene and tetraene. By comparison of the absorption coefficients at these wavelengths with the reference values obtained on purified conjugated materials it is possible to make a quantitative calculation of the amount of conjugation produced for each system (4, 7, 8). The obtaining of such detailed information by use of the maleic anhydride reaction would be extremely difficult.

An application in the study of shortening manufacture has recently become of considerable interest. Lemon (14), and more recently Mattil (15) have presented data which indicate that in the course of hydrogenation of oils containing linolenic acid, such as linseed oil or soybean oil, certain isomeric forms of linoleic acid are produced. These isomeric linoleic acids, which may be produced as the result of the hydrogenation process being initiated at the central double bond of the linolenic system, or as a result of the displacement of double bonds, do not respond to alkali isomerization and do not react with thiocyanogen in the same manner as do the natural fatty acids. Therefore, spectrophotometry does not detect these isomeric materials as linoleic acid. In view of the present interest in the essential fatty acids in nutrition the importance of utilizing spectrophotometry in the preparation of hydrogenated materials should not be overlooked. It can serve as a valuable adjunct to other methods of process control.

Still another application of spectrophotometry is a study of the effect of processing on the double bond systems in oils, somewhat similar to that shown in Figure 5. Figure 7 shows the absorption spectra of cottonseed oil and soybean oil before and after oxidation and bleaching (11, 13). It may be readily observed, in view of the above discussions, that the processing produced increased amounts of conjugated materials. The effects of various bleaching earths have been discussed by Kraybill (11). Investigations of this type are of aid in the evaluation of bleaching materials and their possible effects on keeping quality and flavor reversion.

General

There has been some confusion in the use of spectrophotometric reference constants. For example, the

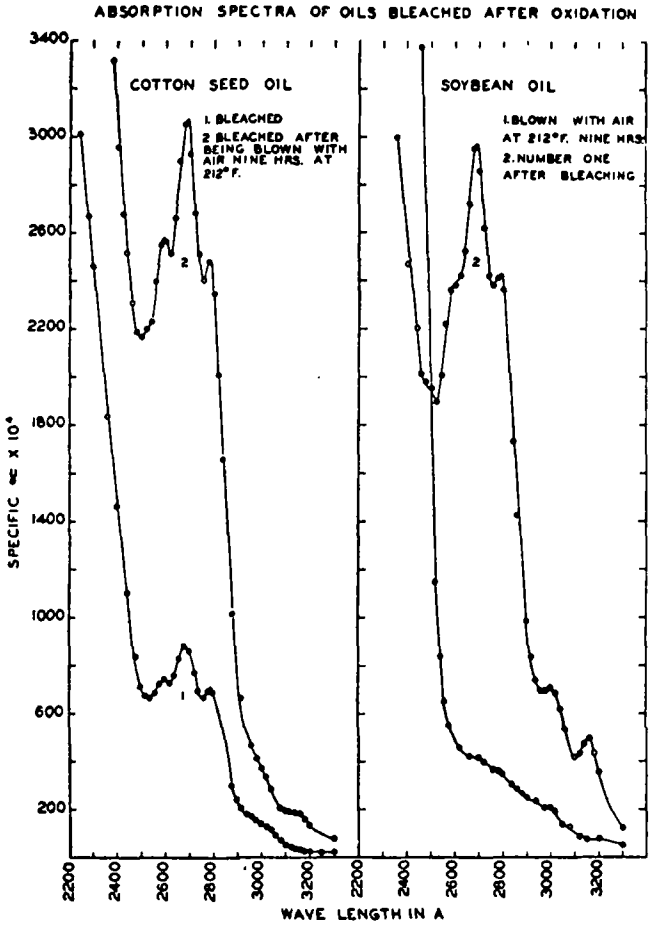

FIG. 7. Ultraviolet absorption spectra of cottonseed and linseed oils bleached after oxidation. [From Mitchell and Kraybill (13).]

reference value for alkali isomerized linoleic acid is about 86.0 while the value for 9-11-octadecadienoic, the all-conjugated form, is about 115.0. The author has observed that there is some uncertainty among inexperienced operators as to the use of these two widely different values. In this connection, the following statement may be helpful: when a fat is to be analyzed for its conjugated diene constituents, the reference constant to be used is that obtained on the purified conjugated material (i.e., the value 115.0); but when a fat is to be analyzed for its linoleic acid content by use of the empirical isomerization method, such as that of Mitchell *et al.*, then the value obtained on empirically treated linoleic acid is the proper reference constant (i.e., 86.0). The same statement would apply to the determination of linolenic and arachidonic acids, as distinguished from their all-conjugated forms.

The explanation for the existence of the different constants lies in the fact that the empirical process of alkali isomerization does not result in a 100% conversion to a conjugated system. The following discussion may further clarify this subject [see Kass and Skell (17)]:

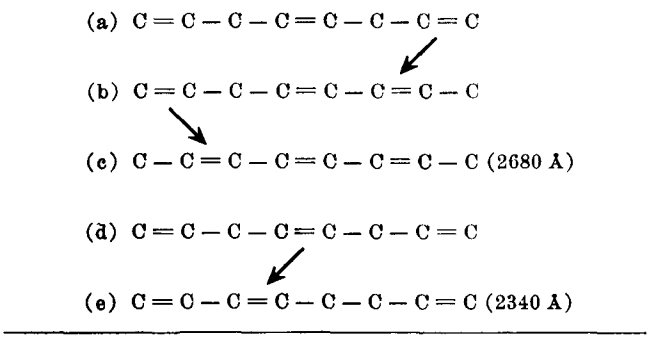

FIG. 8. Some effects of alkali isomerization on the double bond system of linolenic acid.

Line (a) of Figure 8 shows the non-conjugated double bond system which occurs in linolenic acid. Note that each double bond is separated from the nearest neighboring double bond by two single linkages. Now consider that the alkali isomerization treatment produces a triad shift which results in one of the terminal double bonds becoming conjugated with the middle one, as shown in line (b). A subsequent shift of the same sort may then result in the other terminal bond becoming conjugated, as shown in line (c) with the over-all result being the triene conjugation shown in (c). If, however, one visualizes that the first step in the isomerization is not the one mentioned above but is one which results in the central double bond being shifted so as to form a conjugated system with one of the terminal bonds, such as shown in (d) and (e) of Figure 8, the result is that the third double bond is 3 single bonds removed from its nearest double bond neighbor. This is considered to be too great a distance for the triad shift to occur with ease, and therefore conversion of the system shown in (e) to that shown in (c) does not occur. Therefore, linolenic acid yields upon alkali isomerization a diene conjugated system as well as a triene conjugated system, as shown in its absorption spectrum in Figure 3. There are other possibilities of reactions, such as polymerization, which may explain the failure of the linoleic acid to become quantitatively converted to a conjugated system. An extension of the reasoning applied above makes it clear that arachidonic acid would yield not only a four-double-bond conjugated system, but also a three-double-bond and a two-double-bond conjugation, as is borne out by Figure 4. It is essential, then, that the empirical constants be used in the analysis for the non-conjugated acids.

Conclusion

The chemist has in the spectrophotometric method a rapid and simple means of studying changes in the double bond systems of fatty acids. The method is highly sensitive. It has found application in studies of processing of oils, improvement of drying oils, catalytic hydrogenation, routine analytical work, soap and tallow control, as well as in nutrition studies in which the composition of depot and ingested fats are of interest. It also finds application in strictly academic studies which have as their purpose an extension of our knowledge concerning the composition of naturally occurring fats and oils. In general, it is especially valuable in cases in which thiocyanometric procedures are not sufficiently sensitive or in which conjugated as well as non-conjugated constituents occur.

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