Unhydrogenated Soybean Oil

Stability tests on soybean salad oil failed to give the sharp break in P.V.- and butyro-time curves shown (Figure 1) for hydrogenated oils. Rancid odor developed gradually with no readily detectable initial appearance. Figure 2 shows typical P.V. and butyro-time curves for a soybean salad oil.

To establish an arbitrary end-point all previous data on this type of oil was combined in a plot of P.V. vs. butyro change (Figure 3). It was found that a smooth curve was obtained up to the point where the P.V. hit a maximum at about 140 units. From this curve it was found that a butyro change of 1.00 unit corresponded to a P.V. of 85 (the threshold P.V. arbitrarily established for this type of oil in previous runs). Thus by plotting butyro change vs. time for any stability test, the end-point could be established at a butyro change of exactly 1.00 unit. Because of the non-linear nature of the curves determinations of keeping qualities had to be made graphically. Since all samples were run in duplicate, a series of readings from one end of the Swift box to the other gave alternate points for duplicate samples. These were plotted and a mean curve drawn through them to establish the end-point. Figure 4 is the curve for one set of duplicates, showing the type of graph obtained and the method of establishing the end-point.

Accuracy

The degree of accuracy of the method is shown in Table I. From the examination of Figure 4 it is apparent that an error of 0.1 butyro unit (specified maximum for production control work in this laboratory) is equivalent to 0.35 hours, or about 3% of the keeping quality in the case of the particular oil shown. To attain this degree of accuracy, using a refractometer, the reading would have to be reproducible within 0.0001 of a unit. Actually, considerably greater accuracy than this is possible, especially on the easily read Zeiss butyrometer.

Summary

A method is proposed for determining the end-points in the Swift Stability Test by refractive index, which increases the capacity of the Swift box to 18 individual tests and yields well defined curves. The simplicity and accuracy of the method are such as to make it a convenient tool in this test which is rapidly gaining acceptance as a standard. The method is particularly useful in extensive tests using one type of substrate throughout.

Note

An apparent disadvantage of this test is that a correlation must be made between butyro and P.V. for each general type of oil to be tested, to establish a suitable end-point. However, since the test would be run by P.V. anyway, little time is required to measure the butyro of each sample and thus establish the relationship for the type of oil under consideration.

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Spectrophotometric Estimation of Soybean Oil in Admixture With Cottonseed and Peanut Oils[1]

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IT is customary in southern cotton oil mills to process various oilseeds, including soybeans and peanuts, in the same equipment as is used for processing cottonseed. This practice necessarily provides numerous opportunities for one type of oil to become contaminated with another. In one such instance a tank car containing a mixture of cottonseed and soybean oil was shipped to a refiner and was used in the production of shortening without due allowance being made for the unsuspected presence of soybean oil in the original oil. There is always a possibility of accidental, and sometimes unavoidable, contamination from incomplete draining and cleaning the oil troughs, settling tanks, pipe lines, and other processing and handling equipment during conversion from one oilseed to another. Because of the possibility of accidental admixture of soybean with cottonseed and peanut oils and as a means for checking the composition of intentional blending of such oils, a method for estimating the percentage of soybean oil in such admixtures is desirable.

Cottonseed oil may be detected by the Halphen test and peanut oil by its content of higher saturated fatty acids. However, the composition of soybean oil is such that it does not permit its detection or estimation by distinctive color tests or by ordinary chemical methods, especially if it is present in only small proportions. The most distinctive characteristic of soybean oil is its content of linolenic acid which is present to the extent of about 7% in commercial lots. This acid has not been found in cottonseed or peanut oil.

The spectrophotometric measurement of the small but measurable ultraviolet absorption of the triene conjugation, produced by alkali isomerization of linolenic acid or soybean oil, appeared to be the most promising method for the determination of the presence of this oil in mixtures of cottonseed and peanut oils. Mitchell, Kraybill, and Zscheile (4) have described the use of such measurements to determine quantitatively the linolenic and linoleic acid content of various oils. The present authors have established a correlation between the spectrophotometrically measured optical densities of triene conjugation produced by alkali isomerization and the percentage composition of binary oil mixtures. This correlation

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can be applied to the quantitative determination of soybean oil in admixtures with cottonseed, peanut, and similar vegetable oils.

Experimental

The experimental procedure consisted of two steps: the alkali isomerization of the oil mixtures and the spectrophotometric measurement of the optical densities of the isomerized oils at the wavelength position of maximum absorption of the triene conjugation, namely 268 mμ. A modification of the procedure, as described by Mitchell, Kraybill, and Zscheile (4), was used for the alkali isomerization. If a mixture of soybean and cottonseed oils contains 5% soybean oil, which in turn contains about 7% linolenic acid, then the linolenic acid content of the mixture is only about 0.3%. Therefore, it was necessary to modify the procedure of Mitchell *et al.* to adapt it to the measurement of the small quantities of linolenic acid present in such mixtures.

1. The absorption of ultraviolet light by the reagent or blank, when handled by the method of Mitchell *et al.*, was found to be too high to permit measuring absorption of the weak triene conjugation. This limitation was overcome by preparing the reagent and conducting the isomerization under an atmosphere of nitrogen purified by passage through an electrically heated tube containing copper oxide previously reduced by hydrogen. With this modification oxidation of the ethylene glycol in the presence of the alkali is prevented, and a reagent is obtained which is almost completely transparent to ultraviolet light in the region of the triene conjugation. The optical densities of the ethylene glycol-potassium hydroxide reagent, measured through 1 cm. cells against distilled water, are: 220 mμ, 1.89; 232 mμ, 0.357; 250 mμ, 0.092; 268 mμ, 0.063.

2. The entire isomerization was conducted in alkali resistant glassware to avoid the formation of the troublesome white precipitate, described by Mitchell *et al.*, which slowly forms when Pyrex glassware is used.

3. Since the alkali resistant glass would not stand the thermal shock of cooling in cold water, the tubes when removed from the hot isomerization bath were rapidly cooled in a stream of air. To allow for the somewhat slower rate of cooling the time of isomerization was decreased from 25 to 22 minutes.

4. In order to measure the triene conjugation of mixtures containing very small percentages of soybean oil, an investigation was made of the effect of sample size and final dilution of the isomerized oils. On the basis of the results obtained it was found possible to increase the sample size from 0.1 gram to 0.3 gram and to maintain the final dilution at 50 ml., as compared to the 250 ml. recommended by Mitchell *et al.* This modification of the method effected a fifteen-fold increase in concentration and, coupled with the first modification, considerably increased the sensitivity of the method for determining linolenic acid content.

Analytical Procedure

Preparation of Reagent. Seventy ml. of ethylene glycol (E. K. Co.) are placed in a 250-ml. flat bottom flask of Kimble "Resistant" or Corning "Alkali Resistant" glass. The flask is fitted with a cork stopper having an inlet tube for introducing purified nitrogen, an outlet tube, and a thermometer. The inlet tube extends just below the stopper. The outlet tube, which extends to about 10 mm. above the surface of the liquid, is bent so that the emerging vapor stream is directed downward into a graduate where the condensate may be collected. The thermometer bulb is placed below the surface of the ethylene glycol.

A slow stream of purified nitrogen is passed into the flask which is heated on a hot plate until the ethylene glycol reaches a temperature of about 195° C. Four or five ml. are allowed to distill over and condense in the graduate. The hot plate is then removed and the ethylene glycol is allowed to cool to about 100° C. The stopper is raised while 4.5 grams of KOH in pellet form are quickly added. After replacing the stopper, the ethylene glycol is again heated on the hot plate to about 195° C. until a total of 10 ml. of condensate is collected. It is then allowed to cool to room temperature under an atmosphere of nitrogen and is ready to be used.

By this procedure a clear colorless reagent, which has very little absorption in the ultraviolet region, is prepared.

The final alkali concentration is 7.5 grams of 85 per cent potassium hydroxide per 100 ml. of ethylene glycol as recommended by Mitchell *et al. (loc. cit.)*. The nitrogen purification is conveniently accomplished by passing tank nitrogen through a heated combustion tube filled with copper oxide wire which has been previously reduced with hydrogen. The heater is constructed as follows: A standard length of combustion tubing is wrapped with a thin sheet of asbestos 50 cm. long, leaving a 1 cm. gap the length of the tube for observation. Over this asbestos are wound 100 turns of 1/16" x 0.0045" Chromel A ribbon with turns spaced about 5 mm. apart. The tube is then laid in a section of magnesia pipe covering which is arranged so that the top half may be lifted to permit observation of the condition of oxidation of the copper in the tube.

Isomerization of Sample. The isomerization is carried out in approximately 25 x 150 mm. "Alkali Resistant" glass test tubes.[3] Ten ml. of the ethylene glycol-potassium hydroxide reagent are pipetted into each tube, which is fitted with a two-hole cork stopper carrying an inlet tube for purified nitrogen and a straight glass tube about 15 cm. long which serves as an air condenser. The gas inlet tube is extended just through the stopper while the condensing tube is allowed to extend to within about 2 cm. of the surface of the ethylene glycol-potassium hydroxide reagent.

The tubes containing the reagent are kept under an inert atmosphere during heating, isomerization, and cooling by passing a slow stream of purified nitrogen into the gas inlet tube and allowing it to escape through the air condenser. The tubes containing the reagent are placed in a constant temperature oil bath at 180° C. and left for at least 20 minutes to allow the reagent to reach this temperature. One of the tubes of reagent serves as a blank. The stoppers of the other tubes may be raised long enough for a 0.1 to 0.3 gram sample of oil to be dropped in.

The oil samples are weighed in micro-beakers made from "Alkali Resistant" glass tubing. The tubes are shaken three times at one-minute intervals. After twenty-two minutes, the tubes are removed from the bath and quickly cooled by a strong jet of air. It was found that the tubes could be cooled in this manner from 180° C. to 60° C. in about three minutes.

After cooling to room temperature, the samples are transferred to 100-ml. volumetric flasks with the aid of about 30 ml. of 99 per cent ethyl alcohol. They are usually held in a refrigerator overnight as recommended by Mitchell *et al.* (*loc. cit.*), although tests show that this delay in the measurement of the absorption spectra is not necessary when the alkali resistant glassware is used. Although no evidence of precipitation has been observed, as an added precaution, the samples are filtered before absorption measurements are made. Further dilution with 99 per cent specially purified ethyl alcohol is usually necessary, depending on the absorption of the sample. The optical densities of the specially purified alcohol, measured through 1 cm. cells against distilled water, are: 220 mμ, 0.214; 232 mμ, 0.111; 250 mμ, 0.022; 268 mμ, 0.002.

Spectrophotometric Measurements. The spectrophotometric measurements required by the method can be made with either a photographic or a photoelectric instrument. Both instruments were used in this work. This photographic instrument consisted of a high-intensity ultraviolet light source adapted for absorption work in this laboratory (5), a Hilger "Spekker"-type photometer, and a Bausch and Lomb medium quartz spectrograph. The photoelectric instrument used was the Beckman quartz spectrophotometer. By means of either of these instruments the optical density (E) of the isomerized oil mixtures at 268 mμ is measured. From this value the linolenic acid content and the soybean oil content of the mixture are calcu-

[3] Since test tubes of these dimensions made of "Alkali Resistant" glass are not commercially available at the present time, these tubes were made from the necks of long-necked "Alkali Resistant" glass flasks.

lated using equations derived by the methods described under "Discussion."

Discussion

Figure 1 (A and B) illustrates the effect of alkali isomerization upon the ultraviolet absorption of a crude soybean oil. For all the spectrophotometric measurements described in this paper the oils before isomerization were measured in cyclohexane solution with this pure hydrocarbon in the solvent cell. The cyclohexane was purified by methods previously described (3). The isomerized oils were measured in the alcohol-ethylene glycol reagent, with the blank in the solvent cell. The characteristic absorption due to triene conjugation with a maximum at 268 mμ in the spectrum of the isomerized oil is absent in the oil before isomerization. Figure 1 (C and D) shows the effect of an identical treatment on the absorption of a crude cottonseed oil. The cottonseed oil has a measurable general absorption throughout this region which decreases in magnitude with increasing wave length. A comparison of Figure 1 (B) with Figure 1 (D) illustrates the relative effect of alkali isomerization upon the ultraviolet absorption of a crude soybean oil and a crude cottonseed oil.

Figure 1 also illustrates these same effects on a refined soybean and a refined cottonseed oil. From these curves a simple method for the qualitative detection of at least 3 to 4% of soybean oil in cottonseed oil can be readily made. It is necessary only to measure the optical density of the isomerized mixture at 260 mμ and again at 268 mμ. If soybean oil is present in the mixture, the optical density at 268 mμ will be considerably greater than the measurement at 260 mμ (for 268 mμ is a position of maximum absorption of the triene system while 260 mμ is near a position of minimum absorption). If, however, no soybean oil is present in the mixture, the optical density reading at 260 mμ will be greater than the reading at 268 mμ, since the general absorption of the cottonseed oil throughout this region is decreasing rapidly with increasing wave length. (See Table 1.)

TABLE 1

Relative Extinction Coefficients of Vegetable Oils

Oil Sample	Extinction Coefficient	
	a at 260 mμ	a at 268 mμ
Crude soybean oil	0.069	0.070
Crude soybean oil after isomerization	3.34	4.04
Crude cottonseed oil	0.076	0.066
Crude cottonseed oil after isomerization	0.35	0.18
Refined soybean oil	0.16	0.14
Refined soybean oil after isomerization	3.37	4.06
Refined cottonseed oil	0.16	0.14
Refined cottonseed oil after isomerization	0.42	0.27
Crude peanut oil	0.017	0.016
Crude peanut oil after isomerization	0.28	0.18
Refined peanut oil	0.10	0.11
Refined peanut oil after isomerization	0.31	0.21

Usually, however, a quantitative, rather than a qualitative, method for the detection of soybean oil in cottonseed oil is required. Absorption at 268 mμ is due to the presence of the chromophore containing three double bonds in conjugate positions. In the case of soybean oil this chromophore is produced by the isomerization of linolenic acid in the oil during saponification. No similar absorption was expected for isomerized cottonseed oil as chemical tests have not shown this oil to contain linolenic acid. However, it was observed that isomerized cottonseed oil had a definite, though small, absorption at 268 mμ and the absorption of known mixtures of the oils when plotted

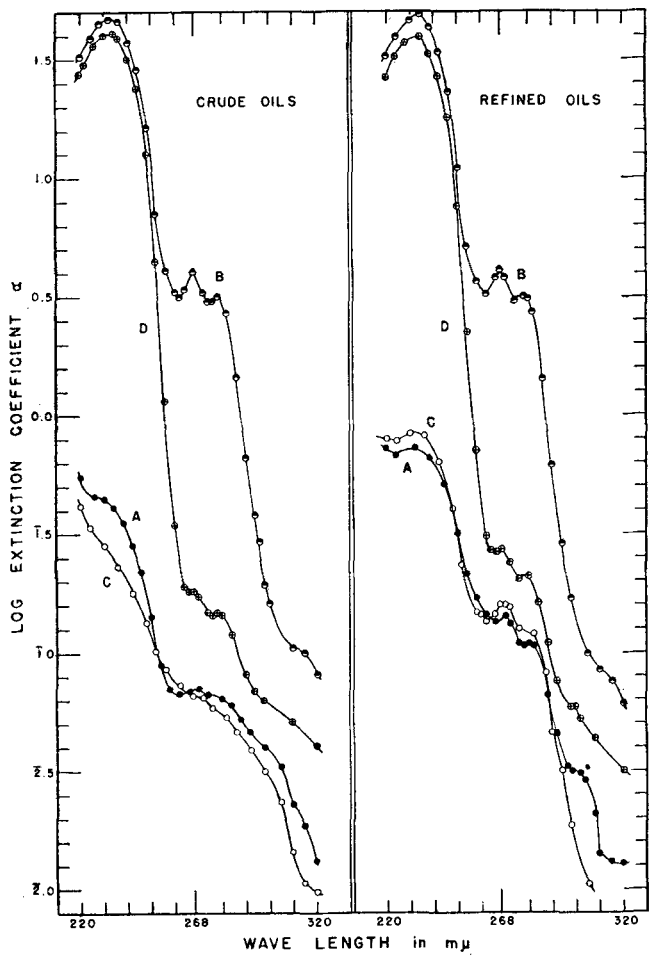

FIG. 1. Ultraviolet absorption of crude and refined soybean and cottonseed oils before and after isomerization.

A. Soybean oil before isomerization.
B. Soybean oil after isomerization
C. Cottonseed oil before isomerization.
D. Cottonseed oil after isomerization.

against the amounts of soybean oil gave a curvilinear instead of the straight line relationship required by the Beer-Lambert law. This absorption constitutes an interference which must be taken into account in applying the principles of spectrophotometry to the determination under consideration.

The most common explanation for the failure of the Beer-Lambert law to apply to a given solution is the presence of an interfering chromophore. The observed behavior is explainable on the basis of an interfering absorption of rather low magnitude which is always included in the optical density measurements. When the soybean oil content of the mixture is high, the contribution of this interfering absorption is relatively negligible. However, when the concentration of soybean oil is low, the contribution of the interfering absorption becomes a major factor in the measurements. This interfering absorption is due either to a small quantity of linolenic acid in the cottonseed oil or to the presence in this oil of some other material which produces triene conjugation upon alkali isomerization.

The character of the low-magnitude absorption is shown by Figure 2. The curves to the left in this figure were obtained by replotting, throughout the limited region of triene conjugation only, the extinction coefficients of the crude and refined cottonseed

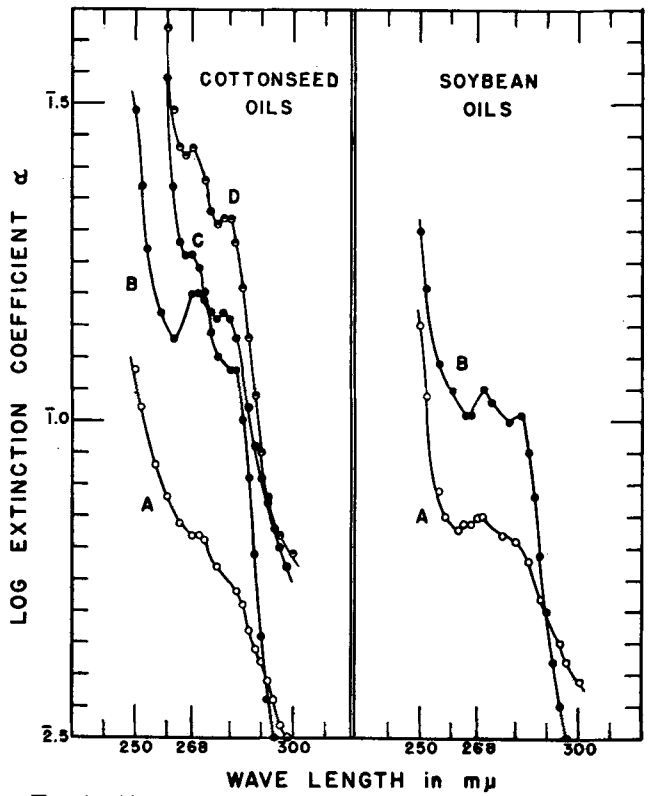

FIG. 2. Absorption of crude and refined cottonseed and soybean oils in region of triene conjugation.
A. Crude oil.
B. Refined oil.
C. Isomerized crude oil.
D. Isomerized refined oil.

oils, both before and after isomerization. The expanded ordinate makes possible a study of the nature of the absorption of the cottonseed oils at 268 mμ. In all four curves the characteristic absorption of triene conjugation is detectable although in the crude oil before isomerization its presence can be observed by only a slight shoulder in the absorption curve at 268 mμ. The refined oil has an appreciably greater absorption in the region of triene conjugation, and isomerization increases this absorption in both the crude and the refined oil. It should be noted that all the absorption values are low. The extinction coefficient range of Figure 2 is only from $a = 0.03$ to $a = 0.4$. The triene conjugated material is, therefore, present in the maximum case of the refined isomerized oil, only to the extent of a few tenths of a per cent. Figure 2 also shows that both the crude and the refined soybean oil have very small but detectable absorption at 268 mμ before isomerization. Again the amount present has increased with the refining processes.

Extinction values for the isomerized soybean oils are, of course, beyond the range covered in Figure 2. Table 1 shows the value of the extinction coefficients at 268 mμ for each of these particular oils. These values vary with the nature and history of the particular oil. From the values for the cottonseed oil it is apparent that for a precise method of determining soybean oil content from optical density measurements, a means of correcting the measurements for absorption due to triene conjugation unrelated to soybean oil content is required.

Several mixtures of a crude soybean oil and a crude cottonseed oil, with gradually increasing proportions of the former, were prepared. Each mixture, and each individual oil, was isomerized, after which the optical density was measured and the linolenic acid content calculated, using the empirical extinction coefficient of Mitchell *et al. (loc. cit.)*. These calculated values were then plotted against the percentage composition of the mixtures. The results, listed in Table 2, show that the pure cottonseed oil had an absorption at 268 mμ equivalent to the absorption of about 0.3% of linolenic acid. This absorption of a cottonseed oil at 268 mμ, expressed in terms of the amount of linolenic acid which would have an equal absorption, will hereafter be called "the apparent linolenic acid content of cottonseed oil."[4] The linolenic acid content of the soybean oil used was 7.7%. In the mixture containing 5% soybean oil having a 7% linolenic acid content, the linolenic acid contributed by the soybean oil, approximately 0.3%, is just about equal to the apparent linolenic acid contributed by the cottonseed oil. Hence, in such a mixture, direct measurements of optical density should give a value about 100% too high. This effect was noted, for mixtures containing 5% or less soybean oil, in first attempts at correlation between direct measurement of optical density and per cent soybean oil.

TABLE 2

Data Used to Correlate Percent Linolenic Acid, Calculated from Optical Density, with Percent of Soybean Oil in Admixtures with Cottonseed Oil

Soybean Oil	Apparent Linolenic Acid*	Soybean Oil	Apparent Linolenic Acid*
Percent	*Percent*	*Percent*	*Percent*
0**	0.37	49.14	3.89
5.86	0.77	70.30	5.43
14.45	1.34	78.02	5.94
17.28	1.53	91.43	6.95
24.35	2.17	100.00	7.68
37.20	3.05		

* All linolenic acid determinations are averages from three density measurements at 268 mμ.
** Cottonseed oil alone.

TABLE 3

Soybean Oil Content of Soybean-Cottonseed Oil Mixtures from Percent Linolenic Acid—Percent Soybean Oil Relationship and Probable Accuracy in Various Soybean Oil-Cottonseed Oil Mixtures

Sample	Soybean Oil in Sample	Soybean Oil Found			Predicted Range in Values of Soybean Oil in Mixtures**
		Range for Determinations*	Average	Deviation from Amount Present	
	Percent	*Percent*	*Percent*	*Percent*	*Percent*
1	4.92	3.69- 4.13	3.91	—1.0	1.55- 4.75
2	9.1	8.00- 8.02	8.02	—1.1	5.04- 9.10
3	9.1	9.67- 9.71	9.69	+0.59	5.51- 9.50
4	15.1	15.8 -15.8	15.8	+0.70	12.4 -18.0
5	15.1	15.4 -15.7	15.6	+0.45	11.8 -17.2
6	25.0	25.3 -25.6	25.4	+0.45	20.5 -28.0
7	50.3	49.1 -50.3	49.7	—0.60	41.8 -54.3
8	75.25	74.2 -75.1	74.7	—0.60	61.6 -79.5

* Range for two to four determinations.
** Predicted range of values of soybean oil which would be found due to variations in the linolenic acid content of different soybean oils or "apparent linolenic acid" content of different cottonseed oils comprising the mixtures. See Figure 5.

Values for the linolenic acid content of the various mixtures are in excellent agreement with the straight line drawn between the points representing the apparent linolenic acid content of the cottonseed oil and the true linolenic acid content of the soybean oil, as shown by the solid line in Figure 3. As a satisfactory

[4] Although cottonseed and peanut oils have never been shown to contain linolenic acid, it is probable that the absorption of these oils at 268 mμ after alkali isomerization is due to the presence of 0.2 to 0.4 percent of linolenic acid in these oils.

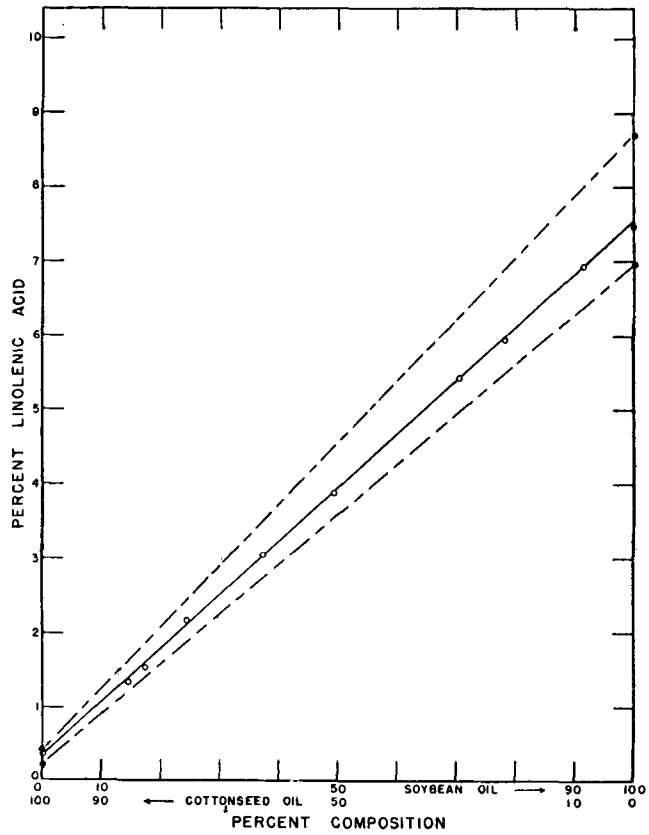

FIG. 3. Working curves for determination of soybean oil in mixtures of cottonseed and soybean oils.

working curve was obtained, it was tested by use in the analysis of a series of "unknowns." The results from this working curve are shown in Table 3. With a known cottonseed oil and a known soybean oil, it is possible to determine the percentage of soybean oil in mixtures to within ± 1%. Calculation of the results from measured optical densities can be reduced to computations from two equations. The percentage of linolenic acid can be computed by use of the Beer's law equation:

(1) Percentage of linolenic acid = E/53.7 x 100/cl. where E is the measured optical density at 268 mμ and 53.7 is the empirical extinction coefficient for alkali isomerized linolenic acid as reported by Mitchell *et al. (loc. cit.)*, c is the concentration of the unknown oil sample in the solution measured expressed in grams per liter and l is the length of the absorption cell in centimeters. With this value for the percentage of linolenic acid, the percentage of soybean oil in the mixture can be computed using the equation of the empirically determined working curve, as illustrated by the compositional diagram of Figure 3:

(2) Percentage of soybean oil =

$$\frac{(\text{Per cent linolenic acid} - 0.33)}{0.0735}$$

where 0.33 is the average value of "y" intercept, and 0.0735 is the slope of the line. Equations (1) and (2) can be combined to the single relationship:

(3) Percentage of soybean oil = $\frac{100\,E - 17.72\,cl.}{3.947\,cl.}$

The accuracy of the method will depend, in large part, upon the accuracy with which the constants of the straight-line equation are obtained. These constants will, in turn, depend upon the variations in the apparent linolenic acid content of various cottonseed oils and the linolenic acid content of various soybean oils.

In order to evaluate the magnitude of possible error in the method due to variations in either the apparent linolenic acid content of cottonseed oils or the true linolenic acid content of soybean oils, the ranges for such values were determined for a number of different crude, refined, and edible cottonseed and soybean oils. The results of some of these measurements are shown in Table 4. The absorption of the cottonseed oils corresponded to an apparent linolenic acid content of from 0.2 to 0.4 per cent. The commercial soybean oils varied in linolenic acid content from 6.8 to 8.7%.

TABLE 4

Linolenic Acid Content of Various Oils from Optical Density Measurements at 268 mμ

Oil Number	Type of Oil	Iodine Number	Linolenic Acid	Apparent Linolenic Acid *
		(Wijs)	*Percent*	*Percent*
S-1	Soybean, crude	131.3	7.47	
S-2	Soybean, refined	134.0	7.64	
3-3	Soybean, refined	134.1	7.98	
S-4	Soybean, crude	132.2	7.14	
S-5	Soybean, crude	134.5	8.71	
S-6	Soybean, refined	132.8	6.97	
S-7	Soybean, crude	129.7	6.08	
C-1	Cottonseed, crude	111.4		0.33
C-2	Cottonseed, refined	103.7		0.42
C-3	Cottonseed, crude	107.4		0.27
C-4	Cottonseed, refined	107.8		0.33
C-5	Cottonseed, refined	103.9		0.30
C-6	Cottonseed, refined	105.3		0.28
P-1	Peanut, crude	94.8		0.25
P-2	Peanut, refined	94.4		0.40
P-3	Peanut, crude	92.1		0.36

* Optical density at 268 mμ expressed in equivalent percent of linolenic acid.

To evaluate the maximum error which could be caused by the measured variation in the cottonseed oils and soybean oils, the soybean oil having the highest linolenic acid content and the cottonseed oil having the highest apparent linolenic acid content were selected and used for plotting another working curve. A similar curve was plotted from the lowest values for each oil. These two curves are shown by the broken lines in Figure 3. From these curves it is possible to calculate the maximum range over which the soybean oil content of the "unknowns" already measured could vary due to differences in the soybean oils and cottonseed oils which could compose the mixtures. Results from some of these calculations are shown in the last column of Table 3.

If the soybean oil content of the mixture is less than 25%, the maximum range with extreme variation in the constituent oils would be from 2 to 3%. Above 25%, this variation increases rapidly so that when more than this proportion is present in the mixture, accurate estimation of the percentage of soybean oil is impossible, due to the range over which the linolenic acid content of various soybean oils present as components in the mixtures may vary. Below 25%, results which should not vary more than 2 to 3% can be obtained. These variations are the possible extremes. Statistically, it might reasonably be expected that the error in the average mixture would be about midway between the extreme and the probable error when using the ideal curve for the particular mixture—that is, from ± 1 to 2%.

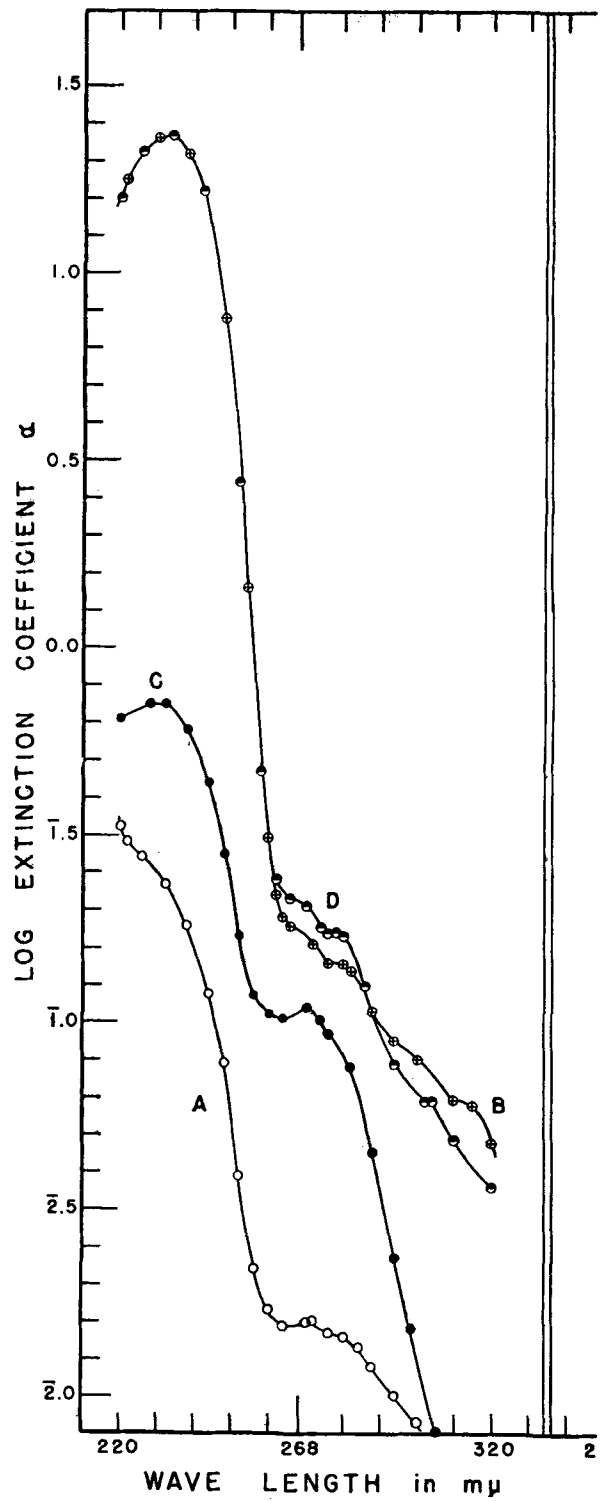

FIG. 4. Ultraviolet absorption of crude and refined peanut oils before and after alkali isomerization.

A. Crude oil before isomerization.
B. Crude oil after isomerization.
C. Refined oil before isomerization.
D. Refined oil after isomerization.

In order to further test the magnitude of error incurred when measuring mixtures of soybean and cottonseed oils having different linolenic and apparent linolenic acid contents, samples containing mixtures of different oils were prepared, isomerized, and measured spectrophotometrically. The results, in Table 5, show that the actual variations are for the most part within the predicted limits.

Two modifications of the method of Mitchell *et al. (loc. cit.)* have been proposed to increase the accuracy of the determination of linolenic acid. Brode, Patterson, Brown, and Frankel (2) suggest a method for correction for diene and tetraene conjugation which interferes with triene measurement, and Brice and Swain (1) propose a method for correction for general background absorption. Neither of these corrections is required for the measurement of the percentage of a particular soybean oil in admixture with a particular cottonseed oil because the magnitude of the interfering absorption is the same in the mixtures used to prepare the working curve and in the unknown mixtures analyzed. These types of corrections need be considered only as variation in the character of the various soybean and cottonseed oils encountered in various mixtures appreciably changes the magnitude of interfering absorption. However, as the magnitude of both of these corrections is very small (1, 2), variations in their magnitude, due to variations in the composition of different mixtures, will be negligible. Hence, only some method, such as the one proposed, of prorating the measured triene conjugation between the true linolenic acid contributed by the soybean oil and the apparent linolenic acid contributed by the cottonseed oil will appreciably improve the accuracy in the determination of the percentage of soybean oil in admixture with cottonseed oil. If, however, a linolenic acid value of the mixture is desired as a criterion of the economic value, use of the corrections as proposed by both Brode, Patterson, Brown, and Frankel (2) and by Brice and Swain (1) should permit a somewhat more accurate estimation of this value.

Measurements of peanut oils showed that they resemble cottonseed oils in that they have an absorption at 268 mμ equivalent to from 0.2 to 0.4% linolenic acid (Table 4, P-1, P-2, and P-3). The absorption of both crude and refined peanut oils before and after isomerization is shown in Figure 4. From these curves, and from the value of the extinction coefficients in Table 1, it will be seen that, like the cottonseed oils, the refined peanut oil had a higher absorption than the crude oil and that isomerization increased the value of the general absorption at 268 mμ (again due, probably, to increase in diene conjugation) without appreciably increasing the triene conjugation. It is possible, therefore, to determine peanut oil in admixture with soybean oil by the method described for cottonseed-soybean oil mixtures. Results of a few determinations are included in Table 5.

Conclusion

A spectrophotometric method has been described for the determination of soybean oil in admixture with cottonseed oil. The method provides a simple and rapid means of detecting gross adulteration of one oil with another and permits an accurate determination of linolenic acid for use as a criterion of the economic value of an oil mixture and as a guide in oil processing.

The factor limiting the precision of the method is variation in composition of the cottonseed and soybean oils in the mixtures to be analyzed. Variations in composition affect the proportion of measured

TABLE 5

Evaluation of Soybean Oil Content of Various Mixtures of Soybean Oils with Cottonseed Oils and Peanut Oils

Oils in Mixtures*			Soybean Oil in Mixture	Linolenic Acid in Mixture (Calculated)	Linolenic Acid Found	Soybean Oil Found	Deviation of Soybean Oil Found from Amount Present
Soybean	Cottonseed	Peanut					
			Percent	*Percent*	*Percent*	*Percent*	*Percent*
S-3	C-4		4.9	0.71	0.68	4.8	—0.1
S-3	C-4		10.0	1.10	1.02	9.4	—0.6
S-2	C-2		7.1	.93	.98	8.8	+1.7
S-3	C-6		15.0	1.40	1.47	15.5	+0.5
S-6	C-4		3.1	.54	.84	6.9	+3.8
S-6	C-6		9.9	.90	1.14	11.0	+1.1
S-1		P-1	7.3	.78	.85	7.1	—0.2
S-3		P-2	10.2	1.18	1.12	10.8	+0.6
S-6		P-2	15.0	1.39	1.50	15.9	+0.9
S-4	C-3		7.0	.75	.82	6.7	—0.3
S-5	C-3		5.0	.69	.80	6.4	+1.4
S-4		P-3	3.0	.56	.70	5.0	+2.0
S-5		P-3	5.0	.78	.83	6.8	+1.8

* See Table 4 for description of oils.

triene conjugation, due to the linolenic acid content of the soybean oil and the apparent linolenic acid content of the cottonseed oil. Thus, for unknown mixtures only average value corrections can be made for apparent linolenic acid content and the accuracy of a particular analysis will depend upon how well the composition of the oils in the particular mixture follows those of the average mixture.

The method described can be extended to mixtures other than those of soybean and cottonseed oils. Thus, soybean oil may be determined in admixture with a peanut oil. In general, any oil which has an unsaturated fatty acid capable of producing triene conjugation upon alkali isomerization can be determined in the presence of any other oil containing no appreciable quantity of unsaturated fatty acids which can produce triene conjugation by such treatment.

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Buttonweed Seed Oil A Source of Linoleic Acid

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BUTTONWEED is listed as one of the secondary noxious weeds of the state of Iowa. The botanical name is *Abutilon theophrasti,* and it is a member of the *Malvaceae* family. It is also known as velvet weed and butterprint. The weed is an annual, but its seeds retain their vitality in the soil so long that many think it is perennial. Mature plants are frequently six feet high with few branches. The seeds are borne in a capsule, shaped like a butter print.

In fields where its growth is abundant the plant is of uniform height of about four to five feet. The seeds are among the most abundant which are screened out of soybeans and are frequently sold to feed companies along with other screenings to be ground into feed.

One of the best ways of controlling this weed, which is becoming a serious menace to crops, would be to collect the seed along with the soybeans and subsequently separate them for extraction of the oil. At the present time an average size seed cleaning plant will produce about five tons of buttonweed seeds during a season.

A search in the literature revealed that Jolson (1) had made a brief study of some of the properties of a cultivated variety of Abutilon seed. No attempt was made to obtain a component analysis of the oil, however. The statement is made that the oil is similar in all its properties to cottonseed and soybean oils.

Experimental

Extraction. Buttonweed seeds for the present study were collected in the vicinity of Newton, Iowa, and were ground in an attrition mill to prepare them for extraction. The oil was extracted with hexane in a large modified Butt extractor. The main portion of the solvent was removed from the oil by straight distillation. Last traces of solvent were removed by bubbling in nitrogen under vacuum. The extracted oil, amounting to 16 to 18%, was dark, yellow green with an iodine value of 130 to 133.

It was observed that seeds obtained at different locations gave oil with a wide variation in iodine value. In one particular field the seeds yielded an oil with an iodine value of 115. The yield of oil from these seeds was somewhat lower, being about 15%.