Conjugated Dienes of Crude Soy Oil: Detection by UV Spectrophotometry and Separation by HPLC[1]

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ABSTRACT

A study of crude soy oils by ultraviolet spectrophotometry showed $E^{1\%}_{1cm}$ values at 233 nm ranging from 1.6 to 2.0. These $E^{1\%}_{1cm}$ values can be used as a measure of the conjugated diene compounds of soy oil. $E^{1\%}_{1cm}$ values for purified triglycerides were 0.78 (tristearin), 1.1 (triolein), and 0.71 (trilinolein). For the triglyceride fraction of crude soy oil after separation by high performance liquid chromatography (HPLC) the $E^{1\%}_{1cm}$ was 0.98. HPLC separation of components in crude soy oil led to a tentative identification of a triglyceride fraction, a carotene fraction, 2 tocopherol fractions, and 4 conjugated diene fractions.

INTRODUCTION

This study is concerned with crude soy oil and how it changes during processing with respect to susceptibility to oxidation and to development of off-flavors. Because hydroperoxides are recognized as the first stable products of oxidative deterioration and the source of off-flavors and because conjugated dienes form simultaneously with hydroperoxides, we have concentrated on studying the conjugated dienes of soy oil and have used ultraviolet (UV) spectrophotometry for the measurement.

Ong (1) used $E^{1\%}_{1cm}$ values at 230 nm as an indication of crude degummed soy oil quality and found that 4 such oils ranged from 1.23 to 1.66 ($E^{1\%}_{1cm}$). UV spectrophotometry and alkali isomerization have been used to determine polyunsaturated fatty acid content of vegetable oils, and in one study, Brice et al. (2) showed that some of the triene and tetraene products are due to autoxidation. Other studies have shown how UV spectra of soy oils change during processing (3-5).

Van den Bosch (6) used $E^{1\%}_{1cm}$ values (based on vol %) to determine deleterious effects of bleaching on soy oil, and St. Angelo et al. (7) used absorbancy at 234 nm as a measure of conjugated diene hydroperoxides in peanut products. DuPlessis et al. (8) used absorbancy at 232 nm to follow deterioration of cottonseed and peanut frying oils. Fishwick and Swoboda (9) described a UV spectrophotometric procedure in which conjugable oxidation products (COP) are detected after reduction and dehydration of an oxidized lipid sample.

In addition to measuring conjugated dienes by UV spectrophotometry, we have experimented with liquid chromatographic separation of conjugated dienes from the triglycerides on silica columns. The separation was monitored by UV spectrophotometry and $E^{1\%}_{1cm}$ values at 233 nm. The results of these experiments are presented and discussed here.

MATERIALS AND METHODS

Soy Oil and Triglycerides

Soy oil samples were obtained from 4 commercial oil extraction companies. Both degummed and nondegummed samples were used, along with one sample of miscella before solvent stripping.

Triglyceride standards were obtained from Sigma (St. Louis, MO) and Nu-Chek-Prep (Elysian, MN).

Spectrophotometry

Most measurements were made with a Varian Model 634S spectrophotometer. All solvents were of high performance liquid chromatographic (HPLC) purity. Hexane was used with small amounts of added tetrahydrofuran (THF) or of isopropyl alcohol (IPA). Some spectra of triglycerides and other oil components were obtained by stopping the pumps on the HPLC unit and using the (Hitachi 155-00) variable wavelength detector with a 20-μL cell and 1-cm path length. Absorbance was measured at 5-10-nm intervals for HPLC peaks and for pure solvent; the solvent absorbancies were subtracted from the peak absorbancies at each wavelength. Holding a sample in 20-μL cell for 7 min showed no decrease in absorbance. Hence, minimal diffusion was occurring during the absorbance readings.

COP Method

The procedure of Fishwick and Swoboda (9) was followed.

Separation Method

Small silica columns (SEP-PAK, Waters Associates, Inc., Milford, MA) were used to separate the conjugated dienes from triglycerides. Based on preliminary experiments with thin layer chromatography (TLC), we chose 1% THF in hexane as a solvent to elute triglycerides and 10% THF in hexane to elute the more polar compounds responsible for increased absorbancy at 233 nm.

Samples of oil (usually 100-500 mg) were placed on the miniature silica columns in hexane and fractions were eluted with successive 10-mL portions of 1% or 10% THF. The eluted samples were examined for absorbancy at 233 nm and a portion was placed in a hot air oven to evaporate the solvent and determine the weight of the sample.

Separation by HPLC was done with a Beckman Model 322 liquid chromatograph. The 150-mm column was packed with 5 μm, spherical particle silica (Ultrasphere-Si, Beckman), and the solvent system was 0.75% IPA in hexane. Solvent flow was 2 mL/min. A filled 20-μL sample loop was used and oil samples were 20% solutions in hexane. Detection was by a variable wavelength spectrophotometer (Hitachi 155-00, 20-μL cell with 1-cm path length) routinely at 233 nm. Other wavelengths used were 225, 267, 295 and 450 nm. For weighing in the μg range, a Cahn Electrobalance Model G was used.

RESULTS AND DISCUSSION

Using the $E^{1\%}_{1cm}$ value, we surveyed crude soybean oils from 4 commercial sources, including degummed and nondegummed oils. Also, from one extraction plant, we obtained miscella to see if the amount of conjugated diene is increased by the desolventizing step. As shown in Table I, the $E^{1\%}_{1cm}$ values ranged from 1.6 to 2.0 for the various crude oils. There was no obvious difference due to degumming or desolventizing. When a sample of crude oil was stored at room temperature for 8 months, the $E^{1\%}_{1cm}$ value increased from 1.6 to 4.6.

Supporting evidence that conjugated diene compounds

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TABLE I

$E^{1\%}_{1cm}$ Values for Commercially Extracted Crude Soy Oils (233 nm in HPLC Hexane)

Oil source	$E^{1\%}_{1cm}$
A-1[a]	1.6
A-1[a] (stored 8 mo, 22 C)	4.6
A-2	2.0
B[a]	2.0
C	1.9
C (miscella)	1.8
D	1.6

[a]Degummed oils.

TABLE II

$E^{1\%}_{1cm}$ Values after Separation of Three Crude Oils on Silica Columns[a]

		Crude oil sample		
		A	B	C
	Initial value	2.3	3.0	7.0
	Fraction 1	1.0	1.2	1.1
1 %	Fraction 2	1.3	1.2	1.2
	Fraction 3	1.5	1.3	1.7
10%	Fraction 4	5.7	8.0	36.6

[a]500 mg of sample on Sep-Pak in hexane; fractions eluted with tetrahydrofuran (THF) in hexane.

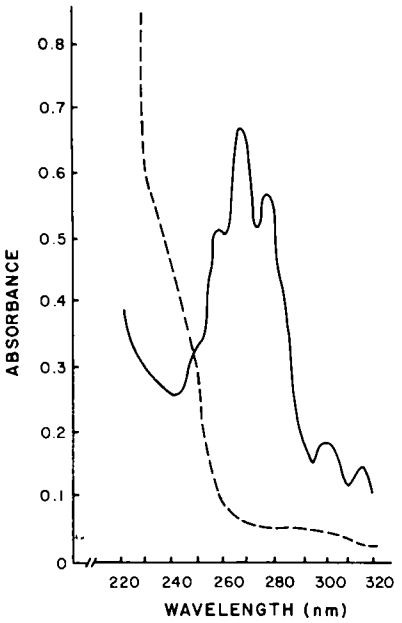

FIG. 1. UV spectra of crude soybean oils. --- in hexane; —— same sample reduced and dehydrated (9).

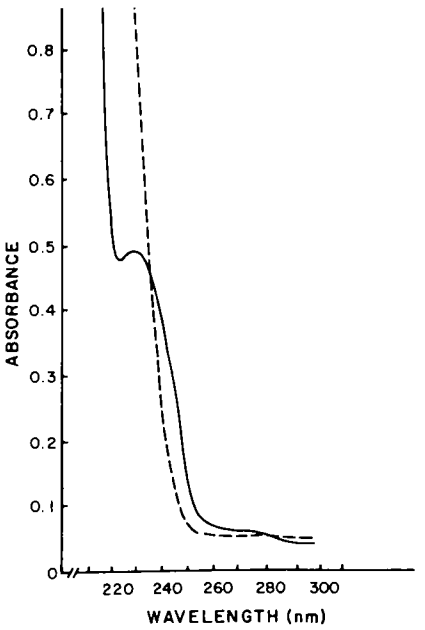

FIG. 2. UV spectra of crude soybean oil fractions separated on a silica column. --- triglyceride fraction with $E^{1\%}_{1cm}$ at 233 nm = 1.0; —— conjugated diene fraction with $E^{1\%}_{1cm}$ at 233 nm = 517.

are present in freshly extracted soy oil was obtained with the conjugable oxidation products (COP) procedure of Fishwick and Swoboda (9). When a degummed crude soybean oil ($E^{1\%}_{1cm}$ 1.6) was reduced and dehydrated, we obtained a definite conjugated triene spectrum as shown in Figure 1. The major change occurred with dehydration. There was relatively little change with reduction (data not shown). The conjugated triene spectrum indicated the presence of conjugated diene compounds in the original crude oil.

Next, we attempted to separate the conjugated dienes in the oil from the unoxidized triglyceride portion. Using information from preliminary TLC experiments, we applied the sample to a SEP-PAK miniature silica column and eluted first with 1% THF in hexane, and then with 10% THF in hexane. Results in Table II show that the fractions eluted from the column with 1% THF were relatively unoxidized, whereas the 10% THF fraction had a much higher $E^{1\%}_{1cm}$ value than the original oil. Figure 2 shows the UV spectra for the 1% and 10% THF fractions of crude soy oil after separation on silica. The 10% THF fraction has a spectrum indicative of conjugated diene.

We noticed that when we attempted to measure $E^{1\%}_{1cm}$ values of soy oils that had been partially hydrogenated, the values were invariably high ($E^{1\%}_{1cm}$ = 23). This resulted from the conjugated dienes produced as a result of hydrogenation. Thus $E^{1\%}_{1cm}$ values are of little use in evaluating the quality of oils or fats that have been hydrogenated.

The range in $E^{1\%}_{1cm}$ values for crude oils in Table I and the wider range achieved by separation on silica (Table II) raised the question of what $E^{1\%}_{1cm}$ values would be for soy oil completely devoid of conjugated diene compounds. As a first step toward investigating this question, we measured the $E^{1\%}_{1cm}$ values at 233 nm for triglyceride standards.

Because tristearin would not be oxidized to conjugated dienes, its absorbancy at 233 nm should be due to the carbonyl group of the ester linkage only. We found the $E^{1\%}_{1cm}$ value for tristearin to be 0.78, and the value for triolein to be 1.1. The reason for the higher value for triolein is unknown; the measurement of $E^{1\%}_{1cm}$ was made on triolein that had been purified by HPLC. Trilinolein standards had an $E^{1\%}_{1cm}$ of 0.71 (the average of 7 determinations with standard deviation of 0.144) when collecting after separation of oxidation products by the HPLC procedure used for soy oil. Polyunsaturated triglyceride standards had evidence of conjugated dienes as received; thus, the separation was necessary to purify the triglycerides. The $E^{1\%}_{1cm}$ value for trilinolein (after separation)

was close to that for tristearin, indicating that the methylene-interrupted double bond system of trilinolein does not absorb appreciably at 233 nm.

The $E^{1\%}_{1cm}$ value for tristearin and trilinolein support the use of 0.07 as a correlation factor (for concentrations of g/L) in the alkali isomerization method of polyunsaturated fatty acid analysis (10).

Separation of soy oil triglycerides from their oxidation products by HPLC gave chromatograms as shown in Figure 3. As THF oxidizes readily in air and could be a confusing factor in separation of oxidized triglycerides, we made use of a hexane:IPA mixture for the HPLC separation on silica.

Most of the sample (>90%) was placed on the HPLC column is eluted in peak 2; hence, we assume that peak 2 is the triglyceride fraction. By collecting the fraction corresponding to peak 2 and by measuring absorbancy at 233 nm and weight, we obtained an $E^{1\%}_{1cm}$ value of 0.98 (average of 9 samples with ± 0.082 SD). This $E^{1\%}_{1cm}$ value is larger than the values obtained from triglyceride standards (except for triolein), and the reason is unknown. There may be UV-absorbing compounds in soy oil that are not separated from the triglycerides by the HPLC procedure and therefore are contributing to the $E^{1\%}_{1cm}$ values at 233 nm. We found, for example, that conjugated triene compounds eluted with the triglyceride fraction.

Calculation of $E^{1\%}_{1cm}$ values for the spectra of Golumbic et al. (3) and O'Connor et al. (5) at 233 nm gave values of 2-3 for crude soy oil. Ong (1) found a range of $E^{1\%}_{1cm}$ values for crude soy oils at 230 nm of 1.23-1.66. Van den Bosch (6) reported $E^{1\%}_{1cm}$ values (230 nm) for crude soy oil of 0.5-0.8 based on vol %. The $E^{1\%}_{1cm}$ based on volume should be roughly 90% of the $E^{1\%}_{1cm}$ based on weight, so that factor alone would not account for the low values reported by Van den Bosch. The $E^{1\%}_{1cm}$ value (233 nm) for triglycerides free of conjugated dienes based on our results was 0.98, and this seems reasonable when compared to other reported values.

For tentative identification of the other fractions separated by HPLC, we made use of previous results of silica chromatography of lipids (11), monitoring the HPLC elution pattern at several wavelengths, and scanning the UV spectra of the fractions while samples were held in the variable wavelength UV detector.

Peak 1, based on its elution position and its absorbancy at 450 nm, is probably due to a carotene. Purified β-carotene eluted from the HPLC column at the same time as peak 1 (evidence not shown).

Peak 3 had increased absorbancy when measured at 268 nm compared to 233 nm; such a change indicated peak 3 was due to an oxodiene. Peaks 4 and 6a have increased absorbancy at 295 nm and their elution times correspond to γ- and δ-tocopherol as found by Carpenter (12) in a similar HPLC procedure.

Figure 4 shows spectra obtained for peaks 4, 6, 6a and 8 when the pumps were stopped with each substance in the detector and absorbance was determined every 10 nm. The evidence from these spectra supported the identification of peaks 4 and 6a due to γ- and δ-tocopherol, respectively. Also, the spectra supported the identification of peaks 6 and 8 as due to conjugated dienes. Peaks 5 and 7 (in Fig. 3) had spectra similar to those of peaks 6 and 8 presented in Figure 4, indicating that they, too, were due to conjugated diene compounds.

These results showed that the crude soy oil immediately after extraction and desolventizing contained conjugated diene compounds and that these compounds could be separated from the triglycerides by liquid chromatography on silica. We intend to pursue this line of investigation by separating sufficient soy triglycerides from conjugated diene components to determine if such triglycerides have increased stability to oxidation compared to the usual crude soy oil.

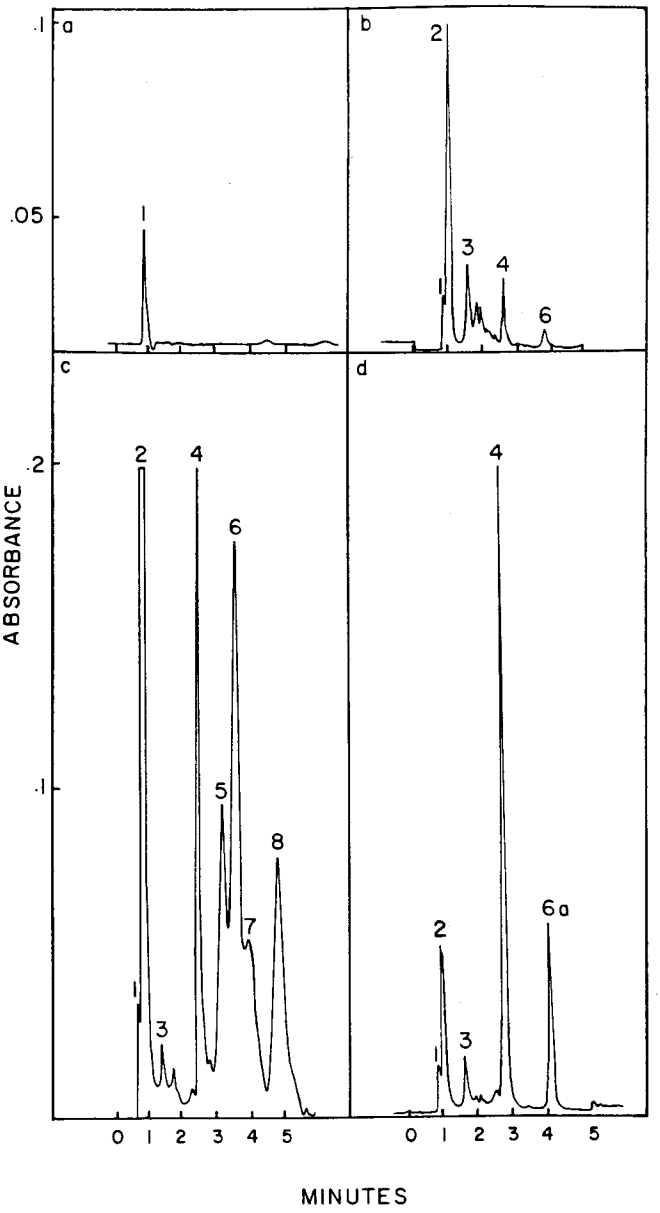

FIG. 3. HPLC separation of crude degummed soybean oil (20 μl of 20% miscella in hexane). Eluting solvent was 0.75% IPA in hexane; 2 ml/min; column, Ultrasphere silica (15 cm × 4.6 nm). (a) 450 nm, (b) 268 nm, (c) 233 nm, (d) 295 nm.

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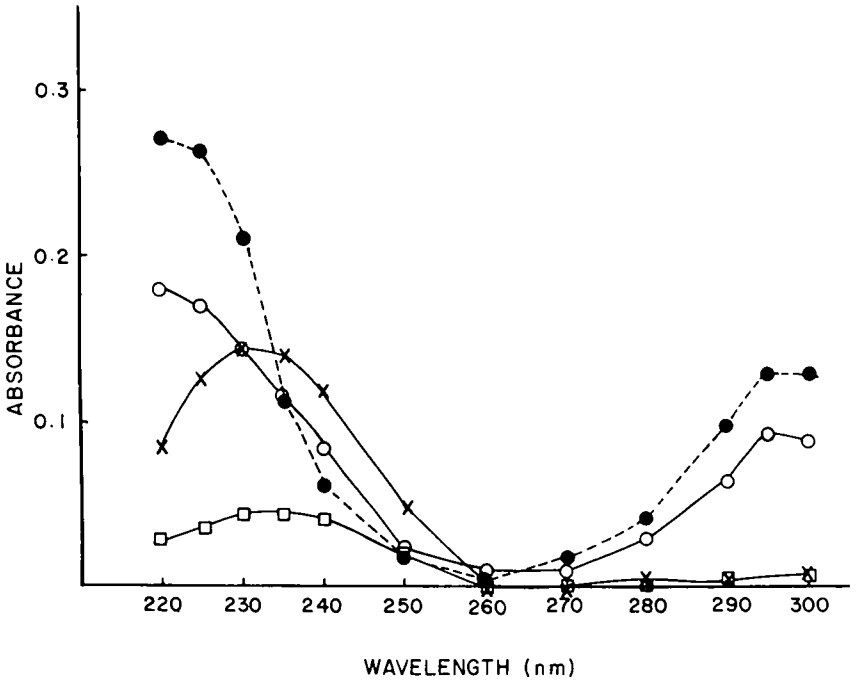

FIG. 4. UV spectra of fractions separated by HPLC. Spectra obtained while fractions were retained in the HPLC detector and numbers correspond to peaks numbered in Fig. 3. ●-● 4; ○-○ 6a; x-x 6; □-□ 8.

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