Cottonseed Oil Pigments: Fractionation by Means of Molecular Sieves, Countercurrent Distribution, and Low-Temperature Crystallization[1]

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Crude cottonseed oils were shown to have spectra of two types, those exhibiting a single maximum at about 360 mμ and those exhibiting a double maxima at about 375 and 400 mμ. Gossypol and cottonseed oil pigments which absorb below about 375 mμ are trapped by molecular sieve No. 13X, appear in the hydrophilic phase in countercurrent distribution of the oil between 95% ethanol and isooctane, and are found in the liquid fraction on crystallization of oil from acetone at −63°C. Those pigments not admitted by molecular sieve No. 13X are found in the hydrophobic phase in countercurrent distribution and in the solid fraction from low-temperature crystallization. Only countercurrent distribution achieved separation of both types of pigments from the triglycerides. Insufficient quantities of pigments were isolated for any extensive characterization. Use of large-scale apparatus would appear to make it possible to separate the pigments for characterization and evaluation of their relation to the refining properties of oils.

INVESTIGATION of a large number of crude cottonseed oils from many different mills has established the fact that no satisfactory correlations exist between spectral properties of the crude oil and Lovibond color of the refined oil (2). Oils were selected to represent a wide range of environmental factors, types of processing, and the effects of storage of both seed and extracted crude oil. While spectral examination of the crude oil may provide useful information, it was not possible statistically to predict with any reliability the refining characteristics, as defined by Lovibond red color.

A further attempt to establish a method for predicting, from simple measurements on the crude cottonseed oil, whether or not it will refine, by the standard refining practices, to a prime-colored oil has been made on the basis of simple fractionations of the crude oil. If a crude oil could, by some simple technique, be fractionated so that the alkali-soluble pigments would be contained in one fraction and the alkali-insoluble pigments in a second, a ratio of the amount of pigments in each fraction might serve as a basis for predicting the color quality of the resulting refined oil. Such fractionation might concentrate the pigments, causing difficulties in refining, so that they would be available in quantities which would permit their further characterization. From such an investigation methods for removing troublesome pigments from the crude oils might be expected. This paper describes methods for the fractionation of pigments of crude cottonseed oils by techniques other than partition or adsorption chromatography.

Experimental

Separation with Molecular Sieves. After preliminary investigation three general methods for separating the pigments in crude cottonseed oils were selected for further study. The first of these consisted of a separation by trapping one pigment fraction on a selected molecular sieve while another fraction passed through the sieve. As rather large molecules are involved, molecular sieve No. 13X, a product of the Linde Air Products Company, was chosen. A chromatographic type of column, 2.3 cm. inside diameter and 25 cm. in length, was filled to a height of 15 cm. with gentle tapping on the side of the column with a rubber mallet to aid packing, with pellet-size 1/16-in. molecular sieve No. 13X. The column was washed with *n*-hexane, b.p. 65–68°C., to remove dust-size particles and then filled to the level of the molecular sieve. Two solutions containing 1 g. of oil sample in 50 ml. of *n*-hexane were prepared, one being used as a control. The second oil-hexane solution was added to the column and eluted with *n*-hexane until the eluant was colorless. The eluant was concentrated to 50 ml. on a steam bath under partial vacuum in an atmosphere of N_2 and carefully brought to a volume of hexane equal to that in which the oil was originally dissolved. Absorption curves of control and molecular sieve eluant from 325 to 700 mμ were obtained with an Applied Physics Recording Spectrophotometer Model No. 14. As the actual pigment concentration is unknown, curves were plotted in relative absorbance units to emphasize qualitative changes only.

Separation with Craig Countercurrent Distribution Apparatus. A second method for fractionating the cottonseed oil pigments was by means of a phasic separation, using a small manually-operated 60-tube Craig Countercurrent Distribution Apparatus. Two parts of isooctane (2,2,4-trimethylpentane), b.p. 99.3°C., were equilibrated with one part of 95% ethanol. The two phases were separated and stored in glass-stoppered bottles until needed. The apparatus, which had a lower phase capacity of 10 ml. per tube, was filled with the stationary lower phase by adding 55 ml. of the equilibrated 95% ethanol to Tubes Nos. 1 and 2, 6 and 7, 11 and 12, etc. This solvent was distributed throughout the instrument by moving the arm to a predetermined spot so that all but 10 ml. were removed from each tube. The excess solvent was caught at the outlet from Tube 59. Ten ml. of the upper phase isooctane solvent was added to Tubes 1 through 6 to insure equilibration of the two phases. A solution consisting of 2 g. ± 0.0001 g. of sample and 10 ml. of each equilibrated solvent was added to Tube 0. The apparatus was shaken gently 50 times to insure mixing of the sample with both phases, the phases were allowed to separate, and then the upper phase was decanted into the next tube. This process was repeated until 60 transfers were completed. The contents of all tubes were removed, and the two phases were separated for further analyses.

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Separation by Low-Temperature Crystallization. The third method selected for fractionation of the pigments involved crystallizations at low temperatures. Ten g. of oil sample and 80 g. of acetone were weighed into a 4 x 18-cm. glass test-tube. The tube was placed into a bath of dry ice and acetone until the sample was below the level of the acetone in the bath. Dry ice was added until a temperature of −63° ± 2°C. was reached. This temperature was maintained for 30 min. A medium porosity fritted-glass funnel was cooled to below −50°C. The sample was filtered through the chilled funnel and washed with 50 ml. of acetone previously cooled to −63°C. The acetone from both fractions was removed by evaporation under vacuum in an atmosphere of N_2 on a water bath at approximately 60°C.

Results and Discussion

Molecular Sieve Experiments. Visible spectra of the oil samples before and after passage through a column of molecular sieve No. 13X were used to indicate fractionation of the pigments. In Figure 1 are shown the spectra of a hexane solution of a refined and bleached cottonseed salad oil containing pure gossypol before and after passage through the molecular sieve column. These spectra show that the gossypol has been quantitatively trapped by the sieve.

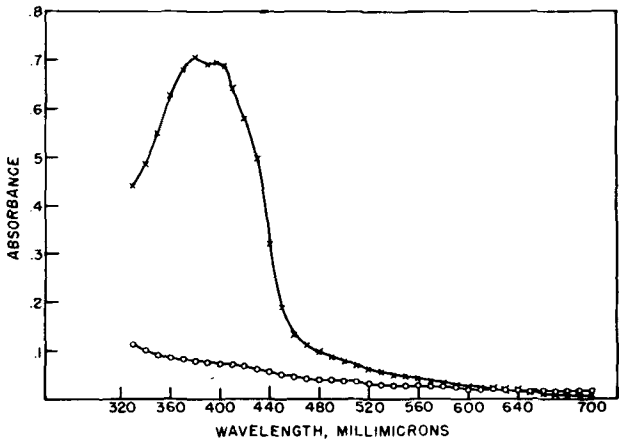

FIG. 1. Absorption spectra of a refined and bleached cottonseed oil containing gossypol: -x-x-x-, before passage through molecular sieve No. 13X; -o-o-o-, after passage through molecular sieves.

The spectra of hexane solutions of six crude cottonseed oils of different Spectral Types (2) and refining characteristics, both before and after passage through the molecular sieve, are shown in Figures 2 and 3. Spectral Types I and II designate oils exhibiting one and two maxima, respectively, in the region of about 360 to 420 mμ. Before passage through the molecular sieve a prepress oil (Table I, oil a), which refined to a Lovibond color of 3.3 red and bleached to 0.6, exhibited a Type I spectrum (Figure 2, A). After passage through the molecular sieve a spectrum (Figure 2, B) which exhibits no maxima whatever through the visible range is observed. In this experiment the pigment giving rise to the characteristic absorption has, like gossypol, been quantitatively removed by the molecular sieve.

The spectra of two additional crude cottonseed oils of Spectral Type I exhibited single absorption peaks in hexane solution at 358 mμ (Figure 2, C) and at 363 mμ (Figure 2, E). The first of these (Figure 2, C) was a prime prepress oil (Table I, oil b) which refined to a Lovibond red color of 4.6. After passage through the molecular sieve a spectrum (Figure 2, D) with a maximum at 370 mμ was observed. The third

TABLE I
Properties of Crude Cottonseed Oils

Oil	Free fatty acids	Gossypol	Iodine value	Refining loss
	%	%		%
a. Prepress	0.5	0.12	110.4	4.6
b. Prepress	1.1	0.41	112.0	6.4
c. Solvent-extracted	0.9	0.38	106.5	4.7
d. Screw-pressed	1.4	0.08	108.5	7.9
e. Slow-break hydraulic	1.9	0.09	111.8	10.4
f. Screw-pressed	11.6	0.23	110.7	36.9
g. Solvent-extracted	2.4	0.63	108.6	9.8
h. Screw-pressed	1.2	0.12	101.8	6.2
i. Screw-pressed	0.9	0.11	110.4	5.4
j. Slow-break screw-pressed	1.7	0.04	100.6	7.8
k. Screw-pressed	4.4	0.23	115.4	15.0

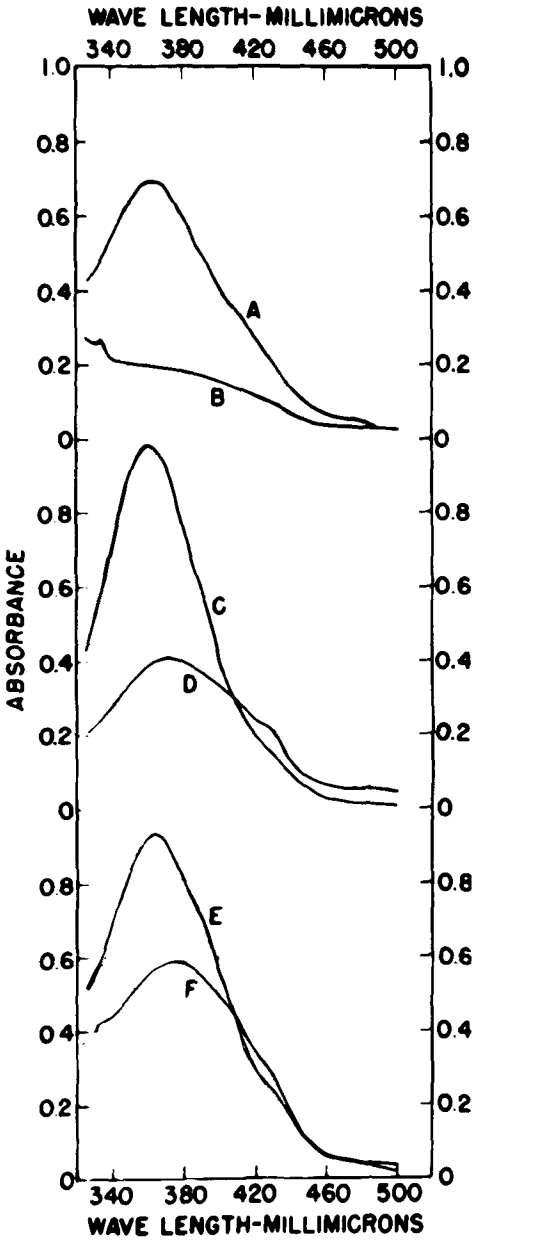

FIG. 2. Absorption spectra of molecular sieves No. 13X fractionated Spectral Type I crude cottonseed oils: A, C, and E, crude cottonseed oils in *n*-hexane; B, D, and F, *n*-hexane eluant of crude cottonseed oils from molecular sieves.

example of a Spectral Type I oil (Figure 2, E) was a solvent-extracted oil (Table I, oil e) that did not refine to a prime color; the Lovibond red color of the refined sample was 20.1. However after passage through the molecular sieve its spectrum exhibited a single band at a longer wavelength, 373 mμ (Figure 2, F).

In Figure 3 are shown examples of the spectra of Spectral Type II oils, in hexane solution before and after passage through the molecular sieve. Figure 3, A is the spectrum of a screw-pressed oil (Table I, oil d) that refined to a prime color, Lovibond red 4.8. After passage of the oil through the molecular sieve the spectral characteristics were unchanged with the two absorption bands at 380 and 400 mμ (Figure 3, B). The oil, the spectrum of which is shown in Figure 3, C, was also unchanged by passage through the molecular sieve, with absorption bands before and after treatment at 382 and 402 mμ. However this oil (Table I, oil e) was a slow-break hydraulic oil which did not refine to a prime color; the Lovibond red color of the refined oil was 9.3.

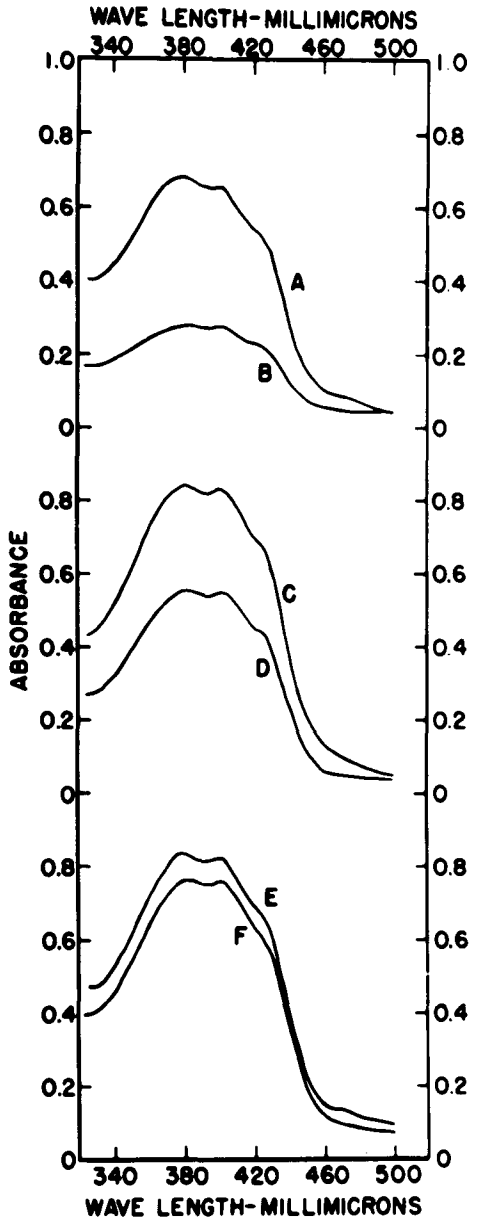

FIG. 3. Absorption spectra of molecular sieves No. 13X fractionated Spectral Type II crude cottonseed oils: A, C, and E, crude cottonseed oils in *n*-hexane; B, D, and F, *n*-hexane eluant of crude cottonseed oils from molecular sieves.

The lower two curves in Figure 3 are spectra of a screw-pressed oil (Table I, oil f) before and after passage through the molecular sieve. This was a very highly-colored oil with a Lovibond red of 20.5. Again there was no qualitative change in the spectra; the original oil and the eluate exhibited two bands at 380 and 396–402 mμ.

On the hypothesis that the exact position of the maximum is a measure of the size of the molecule (*i.e.*, that the larger molecules vibrate with a somewhat lower frequency or higher wavelength) (1), the observed results with the molecular sieve can be explained. Molecular sieve No. 13X will quantitatively trap gossypol from oil in hydrocarbon solution (Figure 1). Apparently this sieve is just the appropriate size, diameter of adsorption cavity 7.41 to 7.52 Å (1), to admit these modified binaphthyl molecules. With the Spectral Type I crude oils the pigments were fractionated by passage of solutions through the molecular sieve. These oils exhibited spectra with a single maximum at about 360 mμ. After passage through the column the spectra either exhibited no characteristic absorption, indicating that the absorbing pigment had, like gossypol, been quantitatively removed by the sieve (Figure 2, B), or exhibited a single band at somewhat longer wavelengths, *ca.* 375 mμ (Figure 2, D and F). The material absorbing, like gossypol, near 360 mμ was completely held by the molecular sieve. The 375 mμ absorbing material is not seen in the spectra of the original oils as it was, presumably, completely hidden by the stronger 360 mμ band. The material absorbing with characteristic maximum at about 375 mμ, it is postulated, consists of somewhat larger molecules as evidenced by the shift to longer wavelength positions of maximal absorption and are too large to be trapped. The intensities are relative and clearly indicate that the material absorbing with a maximum at about 375 mμ was present in considerably lower concentration than the material absorbing at 360 mμ. Two of the oils, the prepress (Figure 2, C and D) and the solvent-extracted sample (Figure 2, E and F), exhibited originally very similar spectra and behaved almost identically on molecular sieves. The eluates from the molecular sieve exhibited very similar spectra even though the first prepress oil refined to a prime color, Lovibond red 4.6, while the solvent-extracted oil sample refined to a very poor color, Lovibond red 20.1.

Similarly (Figure 3) crude cottonseed oils of Spectral Type II exhibiting two bands with maxima at about 380 and 400 mμ were qualitatively unchanged by passage through the sieves. Pigments which absorbed at these longer wavelengths were presumably too large to be trapped by molecular sieve No. 13X. Again there was no qualitative difference in behavior of the screw-pressed oil (Figure 3, A and B) which refined to a prime color, Lovibond red 4.8, and the slow-break hydraulic oil (Figure 3, C and D) which resulted in a poor-colored refined oil, Lovibond red 9.3, or the screw-pressed oil (Figure 3, E and F) which had a very poor Lovibond red refined color, 20.5.

Craig Countercurrent Distribution Experiments. To evaluate the use of Craig countercurrent distribution for separating pigments, gossypol and *beta*-carotene

were dissolved in a refined and bleached cottonseed salad oil. Ethyl alcohol (95%) was placed in the 60-tube Craig apparatus as the lower, heavier, stationary, hydrophilic phase. The oil sample was dissolved in isooctane, and this solution became the lighter or upper mobile hydrophobic phase. At the end of the extraction colored solutions were observed in Tube 7 in the hydrophilic phase and in Tube 48 in the hydrophobic phase. Spectra of these two solutions (Figure 4) show that Tube 7 contained the gossypol and Tube 48 the *beta*-carotene. Separation of the two pigments was quantitative.

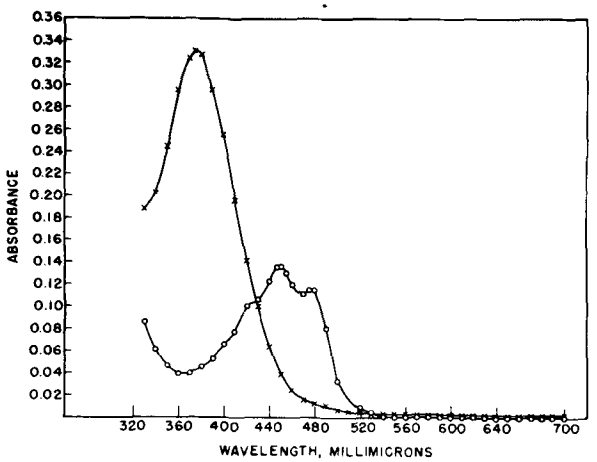

FIG. 4. Absorption spectra of a refined and bleached cottonseed oil containing gossypol and *beta*-carotene: -x-x-x-, transfer Tube No. 7 from Craig countercurrent distribution in 95% ethanol (spectrum of gossypol); -o-o-o-, transfer Tube No. 55 in isooctane (spectrum of *beta*-carotene).

Spectra of the pigments after phasic separation were obtained in the equilibrated solvents, *i.e.*, isooctane equilibrated with ethyl alcohol or 95% ethanol equilibrated with isooctane. In equilibrated isooctane solvent the resulting spectral curve would be nearly identical to that obtained in *n*-hexane or in pure isooctane, and these curves can be compared directly. In the more polar 95% ethanol solvent a bathochromic effect was observed, and the positions of absorption were shifted toward the red or to longer wavelengths. The spectrum of gossypol in the equilibrated isooctane solvent is compared in Figure 5 with that in the equilibrated 95% ethanol solvent. The band at 368 mμ in the hydrocarbon was shifted to 378 mμ in the alcohol. The effect must be remembered in making qualitative comparisons of the spectra in these two solvents.

Table II gives spectral data for six crude cottonseed oils before and after fractionation with the

TABLE II
Spectral Properties of Craig Countercurrent Distribution Fractionated Crude Cottonseed Oils

Oil	Absorption maxima, mμ			
	Original oil	Tubes 0–4, alcohol phase	Tube 7, alcohol phase	Tubes 50–52, isooctane phase
a. Prepress	363	380	377	.....
b. Solvent-extracted	363	376	.....	382
c. Solvent-extracted	363	377	377	377
d. Screw-pressed	380 and 400	373	373	390 and 400
e. Screw-pressed	378 and 400 S	378	.....	385 and 402
f. Screw-pressed	380 and 396–400	380 and 400	.....	382 and 400

Note. "S" indicates shoulder. All other wavelengths are positions of maxima.

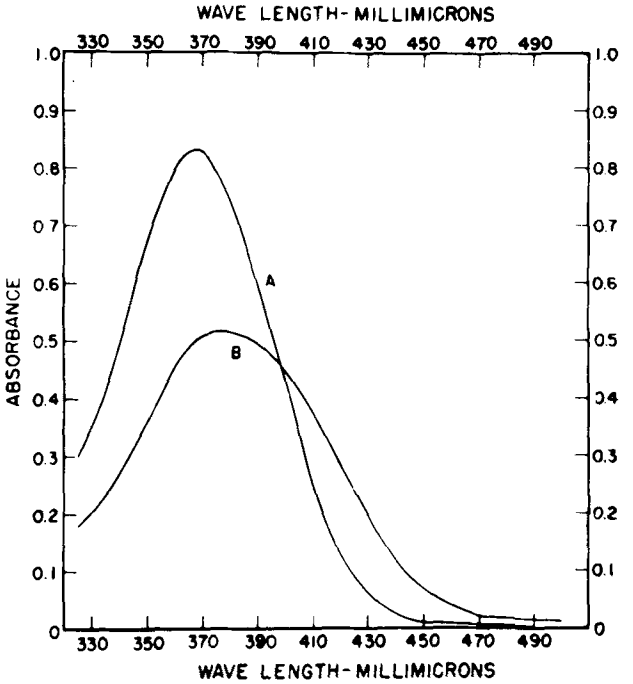

FIG. 5. Absorption spectrum of gossypol: A, in isooctane saturated with 95% ethanol; B, in 95% ethanol saturated with isooctane.

Craig countercurrent apparatus. Three of these oils were of Spectral Type I, exhibiting a single absorption maximum, and three of Spectral Type II, exhibiting two bands at about 380 and 400 mμ.

The first Spectral Type I was a prepress crude cottonseed oil (Table I, oil a) which exhibited a single absorption band at 363 mμ in *n*-hexane solution. It refined to a prime color, Lovibond red 3.3. After phasic separation with the Craig apparatus, pigments were found in the alcoholic phase in Tubes 0–4 and in Tube 7. No pigments were observed in any tubes in the hydrophobic isooctane phase. The pigment in Tubes 0–4 exhibited a spectrum with a single-banded maximum at 380 mμ and that in Tube 7 a single peak at 377 mμ. The second Spectral Type I oil (Table I, oil g) was a solvent-extracted sample which also refined to a prime color, Lovibond red 5.7. The oil exhibited a spectrum, in *n*-hexane solution, with a single-banded absorption peak at 363 mμ. After the phasic separation a pigment was observed in Tubes 0–4 in the alcoholic phase, exhibiting a spectrum with a single-banded maximum at 376 mμ. A second pigment found in the isooctane phase in Tubes 50–58 showed a single absorption maximum at 382 mμ.

The oil (Table I, oil c), the spectrum of which revealed a single-peaked absorption, was also a solvent-extracted oil but with a very high refined color, Lovibond red 20.1. Despite the fact that this oil refined to a very dark color, its spectrum resembled that of the other two prime Spectral Type I oils with a single maximum at 363 mμ. After phasic separation it resembled the very light prepressed oil with pigments observed in the alcoholic phase in Tubes 0–4 with a single maximum at 377 mμ; and a second pigment in Tube 7, the spectrum of which, again like the prepress oil first investigated, exhibited a single band at 377 mμ. The spectrum of the isooctane layer in Tubes 50–58 from this dark oil, like the prime

solvent-extracted sample, revealed a maximum, but the position is at 377 mμ, as found in the hydrophilic phase.

The Spectral Type II oils selected for investigation exhibited very similar spectra with two bands at 380 and 400 mμ. One was a screw-pressed oil (Table I, oil d) which refined to a prime color, Lovibond red 4.8. The other two were screw-pressed samples, neither of which refined to a prime color; one (Table I, oil h) having a refined Lovibond red of 9.3, the other (Table I, oil f) 24.1. After the phasic separations all three of these oils exhibited the same behavior, as shown in Table II, with a pigment concentrated in the alcoholic phase in Tubes 0–4, and a second pigment in Tubes 50–58, in the isooctane phase. Most, probably all, of the oils revealed a weakly-colored distribution peak in Tube 7, the spectrum of which also exhibited a characteristic absorption band with maximum at 377 mμ.

Of particular interest is the distribution of the glycerides in these countercurrent separation experiments. To establish this a quantitative separation was conducted. After the complete phasic separation each of the 120 fractions (60 with the alcoholic and 60 with the isooctane layers) was weighed. The intensity of color in all tubes which showed any evidence of visual color was measured and calculated as absorptivities (based on the weighed contents of the specific tube). The results are shown as a graph of the weight in each tube plotted against the tube or transfer number and as the intensity (absorptivity) of color in each specific tube against tube number for both the hydrophilic and hydrophobic phases in Figure 6.

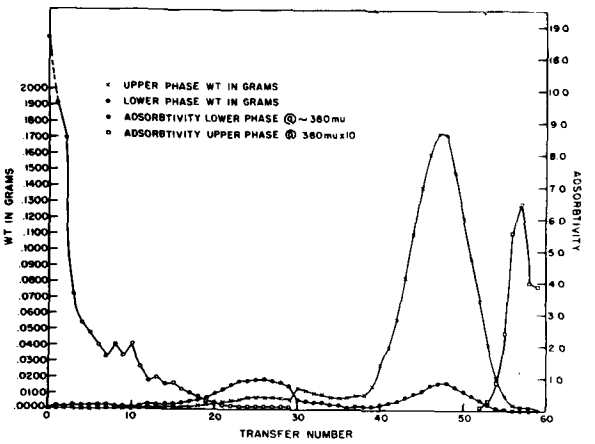

FIG. 6. Weight and color distribution curves from typical Craig countercurrent distribution of crude cottonseed oils in 95% ethanol and isooctane phases.

After distribution the oil was concentrated in Tubes 38 to 55, most of it being found in Tubes 47 and 48. The hydrophobic pigment was concentrated in Tubes 52 to 59, most of it being found in Tube 57. The oil in Tubes 47 and 48 was almost colorless. Thus it would appear that it may be feasible to isolate the pigments from the glyceride oil by countercurrent distribution in the two solvents, isooctane and alcohol. With a somewhat greater number of tubes this separation of the hydrophobic pigment and the oil could be made completely quantitative. The hydrophilic pigment is quantitatively separated from both glyceride and hydrophobic pigment. A system of greater capacity would be required also for isolation of the pigments in sufficient quantity for characterization.

The limitation of sample size, 2 g. of crude cottonseed oil, with the available equipment, has negated all attempts to determine which of the two pigment fractions, the hydrophilic or the hydrophobic, is not alkali-soluble. Sufficient pigment has not been available for refining tests. The countercurrent distribution separation has the advantages that two pigment fractions (and occasionally three) can be recovered without any treatment which would modify them, completely separated from one another and from the glycerides.

Countercurrent distribution separations of a sample of a refined cottonseed oil of high Lovibond red color were attempted to reveal the phasic characteristics of the nonrefinable pigments. The experiment was unsuccessful as, from the 2-g. sample, sufficient pigment is not obtained in any tube to permit determination of its position or elucidation of its spectral characteristics. Repetition of these experiments with considerably higher-capacity equipment might yield interesting results.

TABLE III

Spectral Properties of Low-Temperature Crystallization Fractions of Crude Cottonseed Oils

Oil	Absorption maxima, mμ		
	Original oil	Liquid fraction	Solid fraction
i. Screw-pressed	377 and 400	369	382 and 402
j. Slow-break screw-pressed	380 and 400	372 and 400 S	380 and 400
k. Screw-pressed	380 and 400	371	380 and 402
f. Screw-pressed	380 and 400	376 and 400 S	381 and 402

Note. "S" indicates shoulder. All other wavelengths are positions of maxima.

Separation by Low-Temperature Solvent Crystallization. Four oils were selected for low-temperature crystallization investigations, all of Spectral Type II, exhibiting spectra with maxima at about 380 and 400 mμ but with different refining and bleaching properties: a prime quality screw-pressed oil (Table III, oil i) with a refined Lovibond red of 4.1, a bleached value of 0.9; a slow break screw-pressed oil (Table III, oil j), which refined to a Lovibond red color of 5.9 and bleached to a Lovibond color of 2.3; a screw-pressed oil (Table III, oil k) with a Lovibond red for the refined sample of 8.2 and for the bleached oil 1.6; and a screw-pressed oil (Table III, oil f) with a refined Lovibond red of 20.5 and a bleached color of 6.5. The latter two oils represented samples which refine poorly, one bleaching to a prime bleach color the other to a very highly-colored bleached oil.

As shown by the data in Table III, all four of these samples behaved the same upon low-temperature fractionation. Pigments were found in both the liquid and the solid fractions. The spectra of the pigments in the liquid fractions exhibited mainly a single band between 370 and 375 mμ, at a shorter wavelength than found in the spectra of the original oils. This band is accompanied, in the spectra of two of the four samples, by a very weak shoulder at about 400 mμ. Spectra of solid fractions reveal two well-developed peaks with maxima just above 380 and just above 400 mμ, slightly longer than observed in the unfractionated samples. Thus the shorter wavelength absorbing pigments appear to be concentrating in the liquid phase while the pigments with longer wave-

length characteristic absorption appear to be mainly in the solid fraction. Gossypol, when dissolved in a refined and bleached cottonseed oil, is quantitatively fractionated into the liquid fraction by this technique. As shown in Figure 7, the spectrum of the solid fraction reveals no trace of characteristic absorption.

Comparison of Separation Methods

All three methods of fractionation of the pigments from crude cottonseed oil appear to be primarily separating them into two major fractions. The molecular sieve yielded a pigment fraction which was trapped and one which was not. The countercurrent phasic separations produced a fraction which was preferentially collected in the hydrophilic phase and one which appeared in the hydrophobic phase. The low-temperature crystallization produced a pigment in the liquid and another in the solid fractions. It was of some interest to determine whether these fractionations bear any resemblance to one another. For this purpose a sample of the hydrophilic pigment which was collected in the alcohol in Tubes 0–4 in the Craig apparatus and the pigment found in the hydrophobic phase in Tubes 50–58 were collected and further treated by passage through the molecular sieve. As shown in Figures 8 and 9, the hydrophilic pigment from Tubes 0–4 was quantitatively removed by the sieve while the isooctane soluble pigment was completely unchanged by passage through the column. The ethanol-soluble fraction was thus shown to be similar to the material trapped by the sieve while the isooctane-soluble fraction was the same as that which passed unchanged through the molecular sieve column (Figure 10). Similarly passage of a solution of the solid fraction, from a low-temperature experiment through a molecular sieve, resulted in no apparent change in the pigment while passage of the liquid fraction resulted in almost complete removal of the pigment. So it can be further concluded that the low-temperature solvent crystallization separation divided the pigments into the same two major fractions. The pigment in the solid fraction was the same as that which was not trapped by the sieve or that which was found prefer-

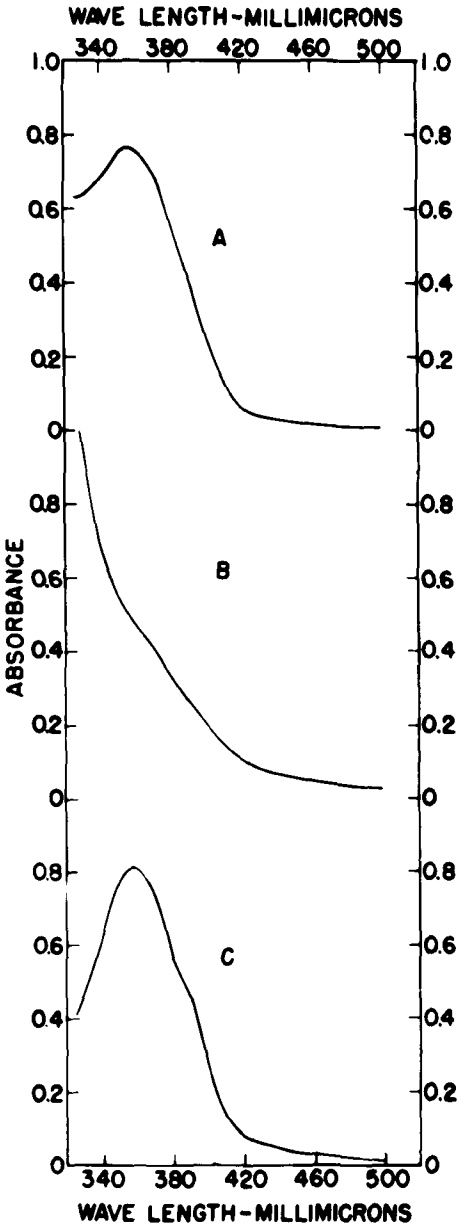

FIG. 7. Absorption spectra of a refined and bleached cottonseed oil containing gossypol: A, pure gossypol in refined cottonseed oil; B, solid fractionation from low-temperature fraction; C, liquid fraction from low-temperature fractionation.

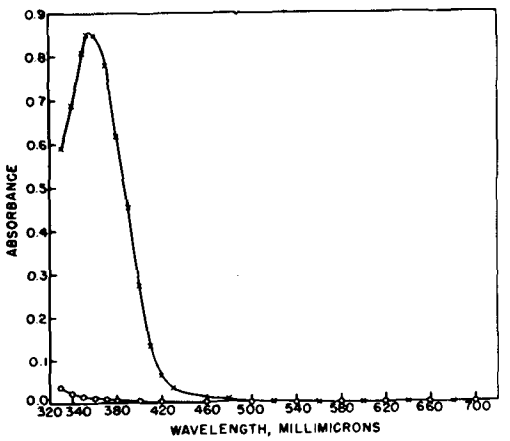

FIG. 8. Absorption spectra of hydrophilic pigment from Craig countercurrent distribution in 95% ethanol: –x–x–x–, before passage through molecular sieves No. 13X; –o–o–o–, after passage through molecular sieves.

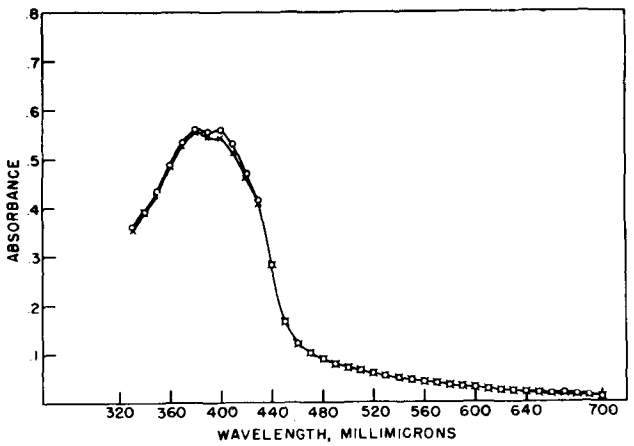

FIG. 9. Absorption spectra of hydrophobic pigment from Craig countercurrent distribution in isooctane: –x–x–x–, before passage through molecular sieves No. 13X; –o–o–o–, after passage through molecular sieve.

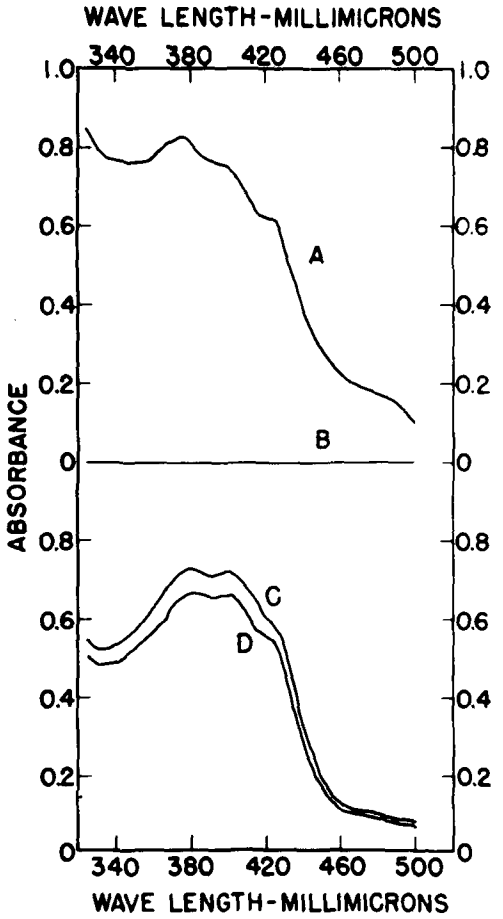

FIG. 10. Absorption spectra of liquid and solid fractions from low-temperature fractionation of crude cottonseed oils: A, liquid fraction before passage through molecular sieves No. 13X; B, liquid fraction after passage through sieves; C, solid fraction before passage through sieves; D, solid fraction after passage through sieves.

entially in the isooctane solvent in the phasic separations. The pigment in the liquid fraction was the same as that which was admitted by the molecular sieve or that found in Tubes 0–4 in the polar solvent in the phasic separations.

Conclusions

Three entirely different techniques have been used to fractionate the pigments of crude cottonseed oil into two major fractions. Molecular sieve No. 13X will trap gossypol and any gossypol derivatives or addition products which are not too greatly different from gossypol in molecular size. In terms of spectral properties the sieve will remove materials absorbing with maxima at or below about 375 mμ but will not remove materials absorbing with maxima above these wavelengths.

Phasic separations between ethanol and isooctane with a small Craig countercurrent distribution apparatus result in two major pigment fractions: one in the hydrophilic phase near the beginning of the distribution and the other in the hydrophobic phase near the end of the distribution. The pigment in the hydrophilic phase appears to possess the spectral properties exhibiting characteristic bands at the lower wavelengths while the pigment which appears in the hydrophobic phase exhibits spectra with characteristic absorption bands at the longer wavelengths.

Low-temperature crystallization fractionation also results in two pigment fractions: the liquid fraction with pigments the spectra of which exhibit bands at lower wavelengths than the unfractionated sample and a solid fraction the spectra of which exhibit bands at wavelengths at slightly longer wavelengths than the original sample.

The three techniques for separating the pigments or crude cottonseed oil appear to be giving identical or very similar pigment fractions. The material trapped by molecular sieve No. 13X appears in the hydrophilic phase in the countercurrent separations and in the liquid fraction in the low-temperature crystallizations. The pigments which are not trapped by molecular sieve No. 13X are found in the hydrophobic phase in the countercurrent separations and in the solid fraction in the low-temperature fractionations. In general, the red pigment content of a refined cottonseed oil could be predicted by a study of the spectral properties of the original crude oils before and after separation by any of the three methods. However these same spectral data will not predict the Lovibond red color.

Separations by means of the Craig countercurrent distribution technique have some advantages over the others. This method of fractionation readily permits both pigment fractions to be recovered for further study and permits a separation of the pigments from the glycerides which is almost quantitative. However, with the apparatus available, only small samples of the crude cottonseed oil could be used, and the quantities of pigments recovered were too small for any extensive investigation to characterize them.

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