A Method for Adsorbent Fractionation of Cottonseed Oil for Experimental Intravenous Fat Emulsions[1]

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Abstract

Results of animal screening tests of emulsified cottonseed oils of the glanded and glandless varieties justify attributing some of the undesirable effects to pigments and minor polar component. A bleaching earth-alumina fractionation method for removal of pigments and polar components of cottonseed oil was developed whereby the triglyceride portion was obtained as an essentially pure, water-white fraction. Bleaching earth and chromatographic alumina when used in sequence were very satisfactory adsorbents, at concentrations of 20 wt % of earth and a 1-to-1 wt ratio of alumina-to-oil. Thin-layer chromatography indicated the presence of sterol esters in the fractionated oil, but no polar components were detected.

Introduction

THERE HAVE BEEN MANY emulsions of cottonseed oil proposed as experimental products for intravenous nutrition. Examples of such products include those stabilized with synthetic emulsifiers (1,2), with soybean lecithin (3), and others. The cottonseed oils in these emulsions, of excellent quality by standards for commercial edible oils, contained pigments and traces of minor components which may be regarded as impurities in a product for intravenous use.

An emulsion of commercial cottonseed salad oil produced more undesirable physiological effects than did an emulsion of commercial soybean oil, the same stabilizer used in each (4). In further studies of emulsions with the same stabilizer, the commercial cottonseed oil was replaced by a refined, bleached, and deodorized cottonseed oil from gland-free seed as the lipid phase, and results of acute tests indicated that the emulsion of gland-free oil apparently was more satisfactory than the emulsion of glanded cottonseed oil. Since gland-free and glanded cottonseed oils show no differences in fatty acid composition (5), the results justify attributing some of the undesirable effects to pigments or color bodies of the glanded oil, and perhaps to its minor components, and point to the desirability of their removal.

The reduction in color in off-colored cottonseed oils by activated alumina has been reported by Pons et al. (6). They found that alumina effectively removed fixed red pigmentation, to produce oils of normal color, but the method was not investigated as a means for removing all of the pigmentation. The effectiveness of adsorbent earths in bleaching menhaden oil was reported (7), but again complete removal of pigments was not attempted.

The object of the present investigation was the development of a method for removing pigments and polar components from normal glanded cottonseed oil as completely as possible, so as to obtain essentially the triglyceride portion of the oil as a water-white product.

Materials and Methods

Oils

One of the cottonseed oils used in this study was obtained from fresh cottonseed by solvent extraction. A second cottonseed oil was obtained from a commercial source as an alkali refined oil. The free fatty acid content of the commercial oil was 0.01% as oleic (8).

Adsorbents for Oil Fractionation

Aluminum oxide was used for column chromatography and was obtained from Merck & Co. (No. 71707, for chromatographic adsorption). The natural bleaching earth used was obtained from Bennett-Clark Co.

Thin-Layer Chromatography

Analysis of the oils by this method employed Adsorbosil-1 (Applied Science Laboratories) as the support, with the developing solvent system petroleum ether-ethyl ether-acetic acid, 90-10-1. Sample concentration was a 10% solution in $CHCl_3$, of which 5 μl was applied. This high concentration of sample was for the purpose of detecting trace quantities of minor components. Spots on the developed plate were visualized by chromic acid spray-charring.

Oil Color

For evaluation of the extent of removal of pigment, or color of the treated oils, visible spectral data were obtained with a Photovolt Colorimeter Model 402E. Percent transmission of the oils was measured at several wavelengths from 390 to 720 mμ. To designate the color of a specific oil sample, per cent transmission at 465 mμ was measured, which wavelength is within the 400–550 mμ spectral range reported to be most useful for the determination of the bleaching activity of clays (7) and is close to the 460 mμ wavelength recommended by AOCS Procedure Cc 13c-50 (8). The light path of the cell used was 150 mm, with distilled water as the reference standard.

Experiment and Results

Cottonseed was dehulled, screened to remove hulls and lint, and the cracked meats fraction, practically hull-free, was flaked to a thickness of 0.008–0.010 in. Oil was extracted from the flakes by soaking with two successive fresh portions of petroleum ether (30–60C) at room temperature, allowing 30 min for each soaking. Miscella was drained from the flakes, and solvent removed by stripping with nitrogen, at reduced pressure and with a minimum of heating (ca. 40C). The crude oil then was alkali refined by the AOCS cup method (8) without heating, washed three times by shaking with distilled water in a separatory funnel, and dried by filtering through Hy-Flo Supercel. Free fatty acid content of the

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crude oil was 0.63%, and of the refined oil 0.02%, as oleic (8).

One kilogram of the extracted, refined oil was dissolved in one liter of petroleum ether, and 200 g of natural bleaching earth was added. The slurry was stirred for 15 min, then filtered; the earth was washed with 200 ml of petroleum ether to remove residual oil. This bleaching of the oil was done at room temperature to lessen the chance of pigment fixation.

Fractionation of the bleached oil was done on a column 8 cm I.D., equipped with a stopcock and a perforated plate for support of the adsorbent. A layer of glass wool was placed on the support plate and covered with Hy-Flo Supercel about 2 cm in depth. One kilogram of alumina as a slurry in ca. one liter of petroleum ether was then added to the column. The bleached oil solution was added to the column and eluted with petroleum ether. Elution of oil could easily be followed by a "heat band" which progressed downward through the length of the column. Elution was continued with two-column volumes of solvent to recover as much oil as possible. Solvent was stripped from the eluate on a rotary evaporator, and the solvent-free oil was then deodorized for 15 min at 225C, 2 mm Hg pressure. Light transmission and thin-layer chromatographic analysis of the oil at various stages of this procedure are given in Table I.

By thin-layer chromatography (TLC) of refined cottonseed oil, spots at R_f values indicative of four significant groups of nontriglycerides were observed below the triglyceride spot: at the origin, and at R_f values of 0.06, 0.10, and 0.20. The locations of these spots indicated the presence of free fatty acids, partial glycerides, and highly polar or oxidation products as determined by known standards (9), and their presence or absence from chromatograms of the oil after various treatments was indicative of the efficiency of removal of polar components. Spots near the solvent front indicated sterol esters, were faint, and were present in all instances. A schematic diagram representing the thin-layer chromatographic analysis of the solvent extracted cottonseed oil is given in Figure 1.

The composition of the oil before treatment, and after the earth and alumina treatment and deodorization, was determined by gas-liquid chromatography, with the following results calculated as peak area:

Fatty acid	Untreated oil, %	Treeated oil, %
Myristic	0.50	0.42
Palmitic	21.45	21.30
Stearic	1.00	1.07
Oleic	12.07	12.69
Linoleic	64.98	64.51

The fractionation method employed appeared to cause no significant change in fatty acid composition.

The visible spectra of the extracted, refined cottonseed oil at various stages of the described fractionation procedure are plotted in Figure 2. Additionally, the results obtained by fractionation of the oil on alumina without prior treatment with earth are included in the figure. It is apparent that the bleaching earth removed those pigments which absorb at lower wavelengths than did the alumina. Treatment of the oil with the combination of adsorbents in sequence was much more effective than either adsorbent alone in the removal of pigments.

TABLE I

Light Transmission and TLC Analysis of Cottonseed Oil at Various Stages of Adsorbent Fractionation

Treatment of oil	Presence of nontriglyceride components at R_f values				Transmission at 465 mμ, %
	Origin	0.06	0.10	0.20	
Crude oil	+	+	+	+	.....
Refined oil	+	+	+	+	0
After earth treatment	+	+	+	+	28.6
After earth treatment and alumina fractionation	—	—	—	trace	91.2
Treated, deodorized oil	—	—	—	—	97.2

Effect of Amount of Earth

The effect of different amounts of bleaching earth on removal of pigments from the extracted, refined oil was determined. In each determination the oil in solution with petroleum ether was stirred with the added earth for 15 min. Light transmission of the solvent-free oil was measured at 465 mμ, and the results obtained are given in Table II. Each portion of oil was then fractionated on alumina, ratio 1:1 as described, and light transmission again measured after removal of solvent. These results, and results of TLC analysis, are included in Table II.

A minimum amount of 15% of earth was required for the significant removal of pigment, and increasing amounts of earth up to 40% showed only minor further removal. After alumina fractionation of the various portions of oil, light transmission greatly increased, with as little as 10% of earth pretreatment, and no polar components were detected. Polar components were not removed by any of the amounts of earth used, but were removed by the alumina fractionation.

Time of Clay Bleaching

Using 20% of bleaching earth in each case, the effect of time of bleaching of extracted, refined cottonseed oil was determined at room temperature. The per cent transmission of the bleached oils at particular wavelengths was measured and the results are given in Table III. A bleaching time of 10 min or less, although somewhat effective in removing pigment, was not as effective as bleaching times of 15 min or more. However, there appeared to be no advantage in exceeding 15 min.

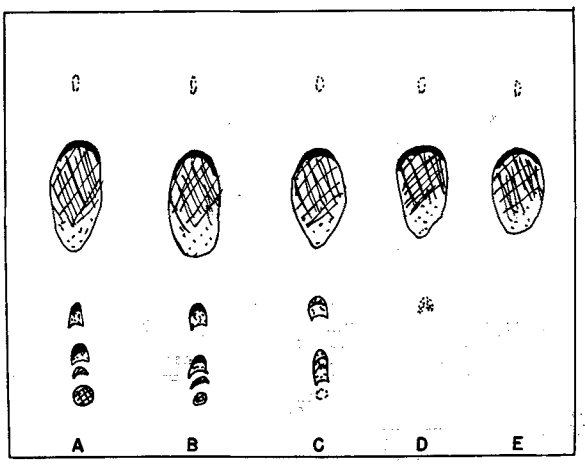

FIG. 1. Schematic thin-layer chromatogram of solvent-extracted cottonseed oil: A, crude oil; B, refined oil; C, refined oil, earth treated; D, refined oil, earth treated and alumina fractionated; E, refined oil, adsorbent fractionated, deodorized.

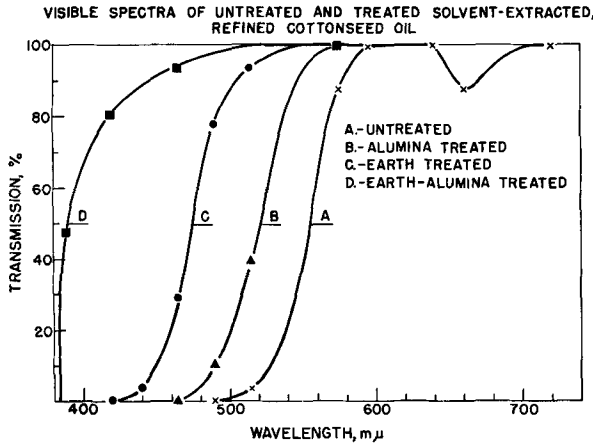

FIG. 2. Visible spectra of treated and untreated solvent-extracted, refined cottonseed oil: A, untreated; B, alumina treated; C, earth treated; D, earth-alumina treated.

Effect of Alumina: Oil Ratio

The effect of variations in the ratio of alumina to oil on the per cent transmission of the resulting fractionated oils was determined at alumina:oil ratios of 0.5:1, 1:1, 2:1, and 4:1, respectively. In each case the oil was first treated with 20% clay, and then added to the appropriate amount of alumina and chromatographed. The results are as follows:

Wt. ratio of alumina : oil	% Transmission at 465 mμ
Original oil	0
0.5:1	35.6
1:1	91.2
2:1	92.0
4:1	92.4

There was no significant increase in removal of pigment at ratios of alumina:oil higher than 1:1. This was the minimum ratio which could be employed for desired pigment removal.

There appeared to be no need for adding antioxidant to the oil fractionated at 1:1 alumina ratio. A small amount (0.05%) of *d*, α-tocopherol was added to a sample of the fractionated oil, and the oil was then stored under nitrogen. Oil without added antioxidant was put in a similar glass container under nitrogen, and both samples were stored at room temperature for five days, under ordinary illumination. The peroxide values (8) (meq O_2/kg) were: oil immediately after bleaching, 1.80; five days in light, 5.38; oil with added tocopherol five days in light, 7.08.

Filtration Chromatography with Alumina

The term "filtration chromatography" has been used to denote the fractionation of cottonseed oil by vacuum filtration on alumina supported on a glass fritted Buchner funnel, rather than by gravity elution on a column. The funnel was prepared with adsorbent in similar manner to the column technique. Extracted, refined cottonseed oil in petroleum ether solution was slurried with earth, filtered, then added to the prepared funnel. Vacuum was applied for filtration. The adsorbent was then washed with petroleum ether, and the washings added to the oil solution. Solvent was removed from the oil, and the oil was deodorized at 225C at a reduced pressure of 2 mm Hg for 15 min. Light transmission at 465 mμ was 87.6%, which indicates that pigment removal was not quite as complete as was obtained by the column method. Polar components, however, were removed.

As a modification of the filtration method, a petroleum ether solution of the solvent-extracted refined oil, previously treated with bleaching earth, was slurried with alumina and stirred for 10 min, then filtered. The transmission of the solvent-free, deodorized oil was 62.9% at 465 mμ, which denotes less pigment removal by slurrying with alumina than by filtration through alumina.

TABLE II

Effect of Different Amounts of Bleaching Earth on Pigment Removal from Extracted, Refined Cottonseed Oil, Followed by Alumina Fractionation

Bleaching earth, %	Light transmission at 465 mμ, %		Presence of polar components	
	Earth bleached	Alumina fractionated	Earth bleached	Alumina fractionated
0	0	40.0	+	−
10	4.0	87.6	+	−
15	25.1	89.1	+	−
20	28.0	92.4	+	−
40	30.2	96.0	+	−

TABLE III

Effect of Time of Bleaching Extracted, Refined Cottonseed Oil on Transmission at Particular Wavelengths

Time of bleaching, min	Light transmission, %					
	420 mμ	440 mμ	465 mμ	490 mμ	515 mμ	575 mμ
5	0	0	15.3	57.6	84.5	100
10	0	4.4	21.5	71.5	86.4	100
15	0	4.3	27.6	78.4	94.0	100
30	0	4.9	25.2	78.9	93.9	100
60	0	6.3	28.3	78.5	91.5	100

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Fractionation of Other Cottonseed Oils

The effectiveness of the described bleaching earth-alumina method in removal of pigments from crude solvent-extracted cottonseed oil and from a commercial refined cottonseed oil was determined. The spectral data of these two oils and of the solvent-extracted refined cottonseed oil for comparison are given in Table IV.

Pigments which absorb at the lower wavelengths were not as effectively removed by the earth, or by the combination of earth-alumina, from the crude solvent-extracted oil and from commercial refined oil as they were from refined solvent-extracted oil. Pigments were more easily removed from crude solvent-extracted oil than from the commercial oil by earth, and by earth-alumina adsorbents. It seems most probable that pigment fixation in the commercial oil was more pronounced than in the solvent extracted oils, crude or refined. Also, refining of the oil prior to the earth treatment was a significant advantage.

TABLE IV

Light Transmission at Particular Wavelengths in Treated Cottonseed Oils

Wavelength, mμ	Transmission, %					
	Solvent extracted oils				Commercial refined oil	
	Crude		Refined			
	Earth	Earth-alumina	Earth	Earth-alumina	Earth	Earth-alumina
390		0		47.5		0
420		19.9	0	80.4		9.6
440		48.1	4.3	90.0		18.5
465		81.9	28.6	91.2	0	50.6
490	0	100	78.4	100	0	92.4
515	0		94.0		0	100
575	68.1		100		49.1	
595	75.4				62.1	
620	88.6				73.1	
640	83.4				79.0	
660	74.8				80.6	
720	100				100	

Bleaching earth adsorbed those pigments which absorb at the lower wavelengths more efficiently than did alumina, at the ratios employed, although neither of these adsorbents alone was as efficient as a combination of the two. Bleaching earth did not remove polar components from the oil, whereas alumina did. This property of alumina was evident in all of the methods of fraction employed, which included column and filtration chromatography, and slurrying.

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