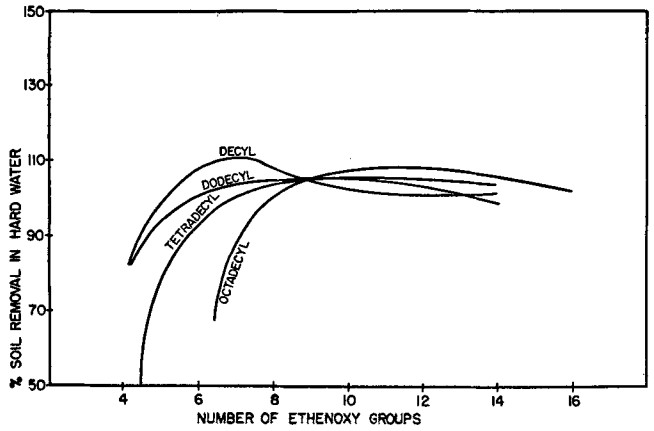

FIG. 3. Variation in soil removal in hard water of polyethenoxy aliphatic ethers with ethenoxy chain length.

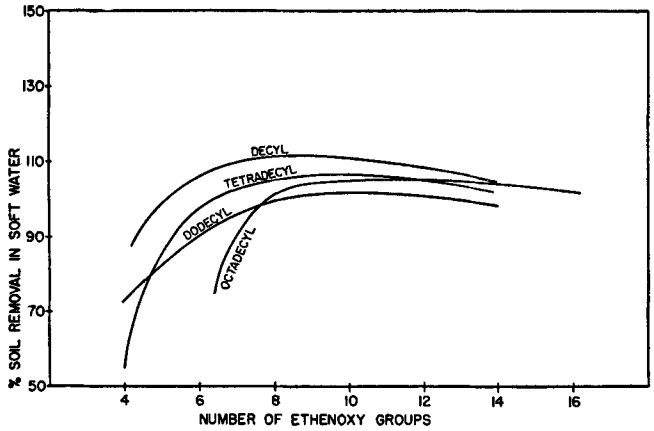

FIG. 4. Variation in soil removal in soft water of polyethenoxy aliphatic ethers with ethenoxy chain length.

point whereupon it levels off or decreases slightly. Here again, the number of ethenoxy groups required for optimum detergency varies with the hydrophobic fatty alcohol chain length and to some extent with each individual detergency test. As in the previously cited experiments (1, 2), two-thirds of an ethenoxy unit for each carbon atom in the hydrophobic unit are required for over-all optimum detergency. In other words, one part by weight of the fatty alcohol or acid should be condensed with two parts by weight of ethylene oxide. It may be also pointed out that the nature of the hydrophobic fatty alcohol portion of the molecule does not greatly influence the detergency values unlike the case of the polyethenoxy alkanoates (1).

TABLE II

Detergency of Polyethenoxy Ethers

Composition of Alcohol Used	Detergency Values of Condensation Product Containing 16 Moles of Ethylene Oxide			
	Whiteness Retention		Soil Removal	
	Hard Water	Soft Water	Hard Water	Soft Water
I.[a] 3.2% Myristyl, 28.4% Cetyl, 19.5% Stearyl, 45.4% Oleyl, 3.5% Linoleyl	129	116	109	107
II.[b] 3.2% Myristyl, 28.4% Cetyl, 68.4% Stearyl	120	117	105	103
Pure Octadecyl Alcohol	120	116	103	102

Sample of [a] Makanol I and [b] Makanol II generously supplied by the Stepan Chemical Company, Chicago, Ill.

Experiments with Commercial Grade Fatty Alcohols

From the data obtained above, it also seemed desirable to select several commercially available long chain alcohols, condense these with 16 moles of ethylene oxide per mole of alcohol, and determine the detergency values of built mixtures in comparison with the polyethenoxy derivative of a purified octadecyl alcohol. The composition of two such commercially available alcohols and the detergency values of their polyethenoxy derivatives in the built mixture are recorded in Table II.

It would thus appear that the presence of some unsaturated fatty alcohol, such as oleyl, in a polyethenoxy ether gives somewhat improved over-all detergency over those containing saturated alcohols alone.

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The Pigments of Crude Cottonseed Oils. I. The Inhibition of Color Reversion in Crude Cottonseed Oils

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PIGMENTS in cottonseed oil have become a serious problem within the past 10 years because of the rapid change from hydraulic to screw-press and prepress-solvent extraction procedures. In the hydraulic mills the cottonseed meats are moistened and cooked at high temperatures, and the oil is extracted at relatively low pressures. Under these conditions nearly all of the pigments remain in the press cake, and there is produced an oil with good refining and bleaching characteristics. In mills which employ low temperature, dry cooking procedures, and high pressures in the presses, a greater percentage of the pigments is extracted and oils more difficult to refine and bleach are produced (1).

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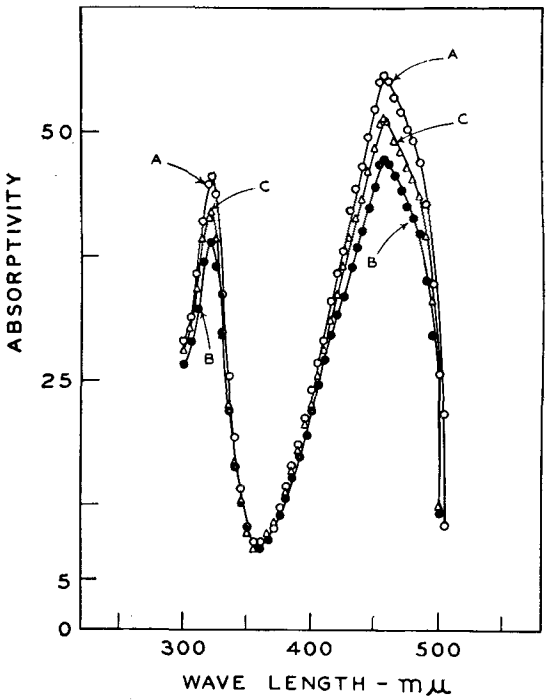

FIG. 1. Di-*p*-carboxyanilinogossypol.
A. "Synthetic" Material.
B. Isolated from Crude Cottonseed Oil.
C. A + B.
(All spectra in anhydrous pyridine)

At the present time representatives of the cottonseed industry have estimated that over 25% of the crude oil now received at refineries cannot be bleached readily to a satisfactory color. Much of this oil would refine and bleach satisfactorily if it were not for the long storage periods at elevated temperatures between processing and refining. During this period some of the pigments change from alkali-soluble to alkali-insoluble forms (color reversion) and are not removed by the conventional refining procedures (2).

A pigment already identified as a component of crude cottonseed oil is gossypol, a polyphenolic binaphthaldehyde. Spectrophotometric data indicate that it occurs in a modified form in the oil or possibly in combination with other components of the oil (1). This modified form and pure gossypol give identical absorption spectra when reacted with *p*-anisidine and can be quantitatively determined by characteristic absorption at 447 mμ (4). The amount of gossypol may vary from less than 0.01% to as much as 1.4%, depending on the original pigmentation of the seed and the processing conditions used to extract the oil (4). In a series of screw-pressed oils color reversion was found to be directly proportional to the amount of gossypol originally present in the oil (3). From these results it would appear that the removal of gossypol from crude cottonseed oils might prevent color reversion.

The objective of this paper is to describe the principles underlying the cause and prevention of color reversion in crude cottonseed oil. The assumption that gossypol is a major factor in color reversion was tested by removing it from crude oils prior to storage by use of a reagent which forms an oil-insoluble compound with gossypol. Analytical and spectrophotometric data are given which afford some insight into the mechanism of inhibition of color reversion.

Experimental

Reaction of Gossypol with p-Aminobenzoic Acid. Aromatic amines which react with aldehydes to form oil-insoluble Schiff bases should be suitable reactants for the removal of gossypol, particularly those amines which contain a carboxyl group, such as the aminobenzoic acids. Schiff bases were prepared from pure gossypol and a number of aromatic amines, including *p*-aminobenzoic acid. The latter served to fulfill most completely the requirements noted above.

In small-scale experiments 200 g. samples of crude cottonseed oil containing about 1% of gossypol were treated with 1.4 g. of *p*-aminobenzoic acid by shaking occasionally and heating in a water bath maintained at a temperature of 75°C. After two hours the precipitation of the orange colored di-*p*-carboxyanilinogossypol appeared to be complete. The composition of this compound, after careful washing successively with petroleum ether, ethanol, and ether and drying in vacuum, is identical with that of a sample prepared from pure gossypol and *p*-aminobenzoic acid in 95% aqueous ethanol, as is shown by elementary analysis:

Calculated for $C_{44}H_{40}O_{10}N_2$: C, 69.83; H, 5.33; N, 3.70. Found: C, 69.28; H, 5.41; N, 3.78 for the "synthetic" Schiff base and C, 69.55; H, 5.52; N, 3.52 for the Schiff base isolated from crude cottonseed oil.

The Schiff base does not melt at temperatures below 350°C., but the absorption spectra of the "synthetic" material and that of the isolated material are identical as are shown in Figure 1.

Treatment of Crude Oils with p-Aminobenzoic Acid. To establish further the principle of "color reversion inhibition" crude cottonseed oils were obtained from six different mills (four screw-pressed, one hydraulic-pressed, and one solvent-extracted oil). Immediately upon collection at the mills 1-gal. sam-

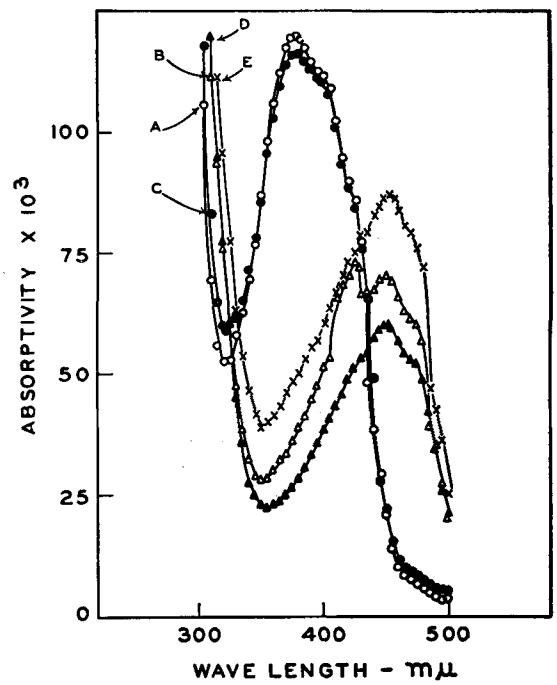

FIG. 2. Crude Screw-Pressed Cottonseed Oil.
A. Control.
B. Treated with *p*-Aminobenzoic Acid.
C. Control Oil Stored for 30 Days at 37-38°C.
D. *p*-Aminobenzoic Acid Treated Oil Stored for 30 Days at 37-38°C.
E. Control Oil Stored for 30 Days at 37-38°C. then treated with *p*-Aminobenzoic Acid.
(All spectra in isooctane solution)

TABLE I.

The Effect of *p*-Aminobenzoic Acid Treatment on the Refining and Bleaching Properties of Crude Cottonseed Oils After 30 Days Storage at 37-38°C.[a]

Type of Oil	Gossypol Before Storage, %	Gossypol After Storage, %	Free Fatty Acid Before Storage, %	Free Fatty Acid After Storage, %	Refining Loss Before Storage, %	Refining Loss After Storage, %	Refined Color[b] Before Storage, %	Refined Color[b] After Storage, %	Bleach Color[b] Before Storage, %	Bleach Color[b] After Storage, %
No. 1; Screw-Pressed	0.65	0.53	1.6	1.6	10.2	10.7	7.2	9.1	2.8	4.3
No. 1 + PABA	0.00	0.00	1.4	1.1	9.7	9.7	8.6	7.0	2.6	2.2
No. 2; Screw-Pressed	0.54	0.42	1.3	1.3	9.3	8.7	5.7	7.5	2.0	2.6
No. 2 + PABA	0.00	0.00	1.1	1.1	6.5	6.2	5.1	4.3	1.7	1.5
No. 3; Screw-Pressed	0.31	0.20	0.9	1.0	5.4	6.6	5.1	5.6	1.3	1.6
No. 3 + PABA	0.00	0.00	1.1	1.0	2.8	1.9	4.8	4.4	1.4	1.0
No. 4; Screw-Pressed	0.25	0.17	1.3	1.4	10.3	10.6	7.4	10.5	2.8	5.1
No. 4 + PABA	0.00	0.00	2.2	2.1	5.0	6.5	6.6	5.1	2.1	1.9
No. 5; Hydraulic-Pressed	0.12	0.07	2.0	1.9	8.0	7.5	6.1	7.6	1.9	3.0
No. 5 + PABA	0.00	0.00	2.4	2.3	4.9	5.2	5.4	4.5	1.7	1.4
No. 6; Solvent-Extracted	0.96	0.75	0.5	0.8	5.6	6.3	12.5	14.1	4.7	14.6
No. 6 + PABA	0.00	0.00	0.5	0.5	1.3	2.9	3.6	3.6	0.7	1.0

[a] *p*-Aminobenzoic Acid (PABA).
[b] Refined and bleach colors were determined by the American Oil Chemists' Society Photometric Method.

ples of the oils were set aside as controls, and identical samples were treated with *p*-aminobenzoic acid. Since the amount of gossypol in the oils could not be determined at the mills, an excess of the reagent (25 g.) was used. The samples were maintained at 75°C. for two hours and with occasional shaking. The samples were then shipped to the laboratory by air express and stored at 3-4°C. until they could be analyzed.

The treated oils were filtered to remove the precipitated Schiff base, and both the control and the treated oils were then analyzed for gossypol, free fatty acid, refining loss, refined color, and bleach color. Portions of both the treated and control oils were stored at 37-38°C. for 30 days, and the analyses were repeated. The difference in red color of the refined and bleached oils before and after storage was used as a measure of color reversion. Gossypol in the control samples was determined by the method of Pons and associates (4) and the absence of gossypol in the treated samples by the disappearance of the characteristic gossypol absorption band at 376 mμ (Fig. 2). Free fatty acids, refining losses, and refined and bleach colors were determined by the official methods of the American Oil Chemists' Society. The results of the analyses are shown in Table I.

As is shown in the table, the treatment removed all of the gossypol from the oils, and there was no increase in refined and bleach color after storage of these oils. That is, color reversion was entirely prevented. Evidently color reversion begins as soon as the oil is extracted since the refined and bleach colors of the treated oils were in most cases lower than those of the control oils even before storage. This was particularly noticeable with the solvent-extracted oil. This oil contained nearly 1% of gossypol and the color of the refined oil was 12.5 red units while that of the treated, refined oil was 3.6 red units. After storage, the control solvent-extracted oil refined to 14.1 units of red color and could not be bleached while the treated oil gave essentially the same refined and bleach colors as were obtained before storage. See Table I, oil No. 6.

After storage of treated, filtered oils a small amount of di-*p*-carboxyanilinogossypol had precipitated. This indicates that the reaction was not quite complete before filtration and accounts for the further decrease in refined and bleach colors observed among treated oils at the end of the storage period. This explanation is substantiated by the increase in the total absorption of the oil in the region of 450 mμ after *p*-aminobenzoic acid treatment (see Curve "B" of Fig. 2).

In addition to color reversion inhibition, the following conclusions can be drawn also from the data shown in the table. Some of the gossypol disappears during storage of the control samples; free fatty acids did not change appreciably throughout the experiment; and refining losses were reduced in all of the treated samples. It is possible that this reduction could be accounted for by the co-precipitation of gums, waxes, and pigments other than gossypol with the Schiff base.

In addition to these studies, others were made on (A) oil stability, (B) the removal of pigments during the alkali refining, (C) treatment of color reverted oils with *p*-aminobenzoic acid, and (D) spectrophotometric examination of the treated and control oils. The stabilities of the stored, bleached oils were determined by the method described by Mehlenbacher (5). No significant difference was found, both treated and control oils required 10.1 to 12.8 hrs. to acquire 100 milliequivalents of peroxide per kilogram.

Treated oils were stored without filtering, and the pigments were removed during the alkali refining procedure. The refined and bleached oils obtained in this manner were lighter in color than those obtained from the control oils, but not as light as those obtained from treated oils which had been filtered prior to storage. This is to be expected since the di-*p*-carboxyanilinogossypol present in the treated oils is subject to considerable hydrolysis and air oxidation when dissolved by the alkaline solutions used in the refining procedure. Portions of the control oils which had been stored at 37-38°C. for 30 days were treated with *p*-aminobenzoic acid and after filtration yielded refined and bleached oils which were lighter in color than the stored controls but darker than the treated, stored oils.

Ultraviolet Spectra of p-Aminobenzoic Acid Treated Cottonseed Oils. Figures 2, 3, and 4 are the absorption spectra of samples of one of the screw-pressed oils (No. 4 of Table I) at each stage of the experiment. The changes occurring in these spectra can be interpreted by reference to previous studies on the absorption of crude cottonseed oils (6, 7, 4). The spectra of crude screw-pressed cottonseed oils in isooctane solutions are exhibited by Figure 2. Curve "A," the spectrum of the original control oil, shows the ab-

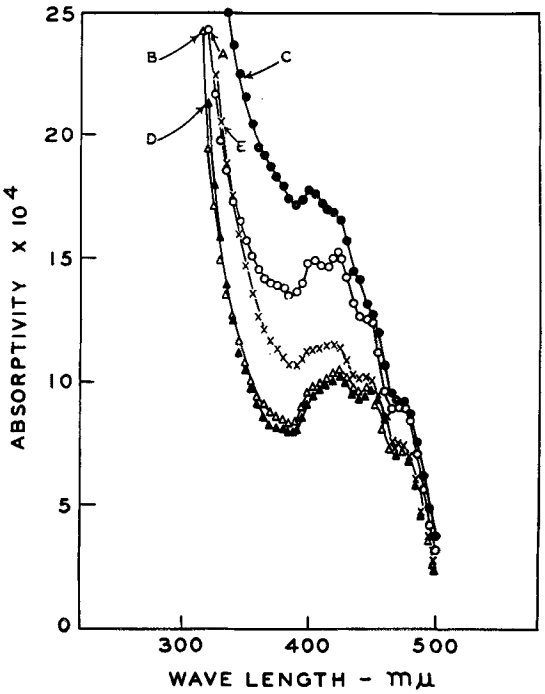

FIG. 3. Alkali-Refined Screw-Pressed Cottonseed Oil.
A. Control.
B. Treated with *p*-Aminobenzoic Acid.
C. Control Oil Stored for 30 Days at 37-38°C.
D. *p*-Aminobenzoic Acid Treated Oil Stored for 30 Days at 37-38°C.
E. Control Oil Stored for 30 Days at 37-38°C. then treated with *p*-Aminobenzoic Acid.
(All spectra in isooctane solution)

sorption band with a maximum at 376 mμ attributable to a gossypol-like pigment which is characteristic of crude screw-pressed cottonseed oils. This band disappears entirely on treatment of the oil with *p*-aminobenzoic acid (Curve "B"), demonstrating the complete removal of the pigment by this reagent. In Curve "B" bands with maxima at 425 and 450 mμ, which are characteristic of carotenoids, are shown. Obviously these bands, masked in the spectra of the control oil by the large amounts of gossypol-like pigments, are now revealed by the chemical treatment as such. Curve "B" thus resembles the spectrum of an alkali-refined cottonseed oil where the bands attributable to carotenoids are revealed as the masking gossypol is removed by the alkali-refining procedure (1). It is to be noted that the total absorption of the oil in the region 450 mμ has been increased by the chemical treatment. The most obvious explanation for this increased absorption is that the carotenoid bands are superimposed on a smooth absorption increasing toward the shorter wave lengths. This smooth absorption must arise from a trace of di-*p*-carboxyanilinogossypol, which is in solution in the treated oil. This explanation is supported by a study of band shapes of other treated cottonseed oils.

Curves "C" and "D" of Figure 2 are similar to Curves "A" and "B," respectively. Curve "C" is the spectrum of the same control crude screw-pressed cottonseed oil after storage for 30 days at 37-38°C., and "D" is the treated oil after similar storage.

Curve "E" of Figure 2 is the spectrum of the crude screw-pressed cottonseed oil, which was stored for 30 days at 37-38°C. and then treated with *p*-aminobenzoic acid. The curve is similar to that of other treated samples (Curves "B" and "D") but the total absorption at about 450 mμ is somewhat greater. This means that either a somewhat greater quantity of di-*p*-carboxyanilinogossypol has dissolved in the treated oil after storage, or the reagent does not remove the gossypol-like pigments as effectively from the stored oil. From spectral evidence alone it would be preferable to treat the crude oil before, rather than after storage.

Figure 3 shows the spectra of the same screw-pressed cottonseed oil after alkali refining. The five spectra represent samples treated in the same manner as the crude oil. As most of the gossypol-like pigments have been removed from the oil by the alkali-refining procedure, the spectra are all quite similar, being characterized by the carotenoid bands with maxima at 425 and 450 mμ. Two conclusions become apparent upon closer inspection of Figure 3. The chemical treatment removes more of the gossypol-like pigments than the alkali-refining procedure. The spectra of the treated oils before and after storage (Curves "B" and "D") reveal less absorption than those of the control oils (Curves "A" and "C"). As the treated samples undoubtedly contain a trace of the di-*p*-carboxyanilinogossypol, their absorption would be expected to be increased in the carotenoid region, as in the case of the crude oils. The higher absorption of the control samples must be attributed to incomplete removal of the gossypol-like pigments. The spectra of the sample of oil treated with *p*-aminobenzoic acid after storage exhibits a somewhat higher absorption than the sample treated before storage, again indicating either more reaction product in the oil or less complete removal of the gossypol-like pigments. In either case, spectral data indicate that treatment of a crude cottonseed oil with *p*-aminobenzoic acid before storage is more effective than treatment

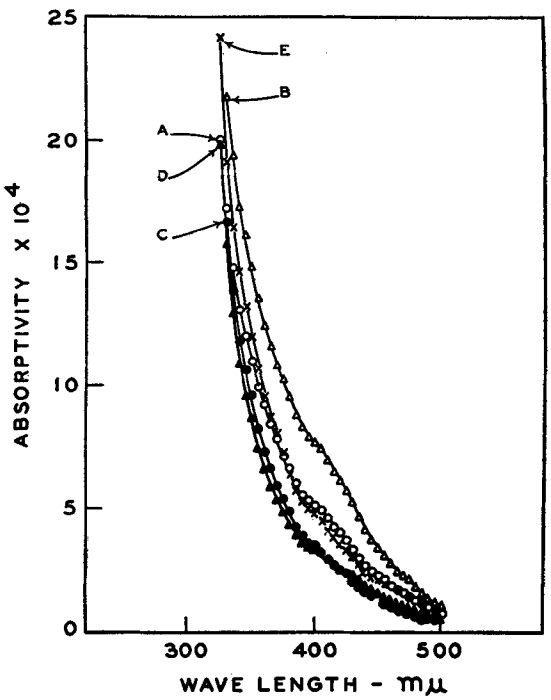

FIG. 4. Alkali-Refined and Bleached Screw-Pressed Cottonseed Oil.
A. Control.
B. Treated with *p*-Aminobenzoic Acid.
C. Control Oil Stored for 30 Days at 37-38°C.
D. *p*-Aminobenzoic Acid Treated Oil Stored for 30 Days at 37-38°C.
E. Control Oil Stored for 30 Days at 37-38°C. then treated with *p*-Aminobenzoic Acid.
(All spectra in isooctane solution)

after storage, as was concluded also from a study of the spectra of treated and control crude oils.

Bleaching removes all traces of carotenoids, and there is no significant difference in the spectra of the treated and the control screw-pressed cottonseed oils after alkali-refining and bleaching (Fig. 4).

In this study the carotenoids of the cottonseed oils have not been identified, but the *p*-aminobenzoic acid removes interfering pigments to such an extent that the treated oils should serve as an excellent source of material for further studies on these yellow pigments normally found in crude cottonseed oils. Preliminary experiments with the objective of separating and identifying the carotenoids of such a treated cottonseed oil have been already initiated in this laboratory.

Discussion

The use of *p*-aminobenzoic acid to precipitate gossypol from freshly prepared crude cottonseed oils prevents color reversion on storage of these oils under conditions which promote color reversion. The fact that gossypol was precipitated by the reagent was proved by the recovery of a gossypol derivative, di-*p*-carboxyanilinogossypol, from the preciptate and by spectrophotometric data. Other materials of unknown nature are co-precipitated by this treatment, but their role in color reversion has not been established. These findings establish the principle of the cause and prevention of color reversion in crude cottonseed oil.

This work suggests an effective approach to the inhibition of color reversion during the storage of crude cottonseed oil. In order to be practical however the reagents used to precipitate the gossypol must be economical or the gossypol derivative must have sufficient value of its own to pay for the extra cost of treatment.

Summary

Treatment of freshly prepared crude cottonseed oils with *p*-aminobenzoic acid and subsequent removal of the di-*p*-carboxyanilinogossypol formed makes it possible to store the oils at a relatively high temperature (37-38°C.) and for an extended period of time (30 days) without incurring any adverse changes in the refining and bleaching properties of the oils. In addition, a considerable decrease in the refining loss of the crude oil is obtained, and the stability of the bleached oil is not affected by the treatment.

Spectrophotometric studies made during all phases of the chemical treatment and during the refining and bleaching procedures show that the *p*-aminobenzoic acid removes almost completely the gossypol-like pigments which are present in the crude oils and yields oils having the characteristic carotenoid spectrum.

Acknowledgment

The authors wish to express their appreciation to Lawrence E. Brown of the Analytical Section for performing the microanalyses, and to Donald Mitcham of the Physics Section for the preparation of the absorption spectra.

The cooperation and assistance of the following oil mills in supplying samples of freshly prepared crude cottonseed oils are gratefully acknowledged: Western Cotton Oil Company, Lubbock, Tex.; Union Oil Mill, West Monroe, La.; Red River Cotton Oil Company Inc., Alexandria, La.; Brazos Valley Cotton Oil Company, Waco, Tex.; and Plains Cooperative Oil Mill, Lubbock, Tex.

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Analysis of Butylated Hydroxyanisole in Paper and Paperboard

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THE use of butylated hydroxyanisole (BHA) in paper and paperboard used for packaging fats and oils, and in foods containing fats and oils has made a method of analysis necessary. This discussion includes qualitative and quantitative methods for BHA in paper and paperboard. A new method for the rapid quantitative determination of BHA in paper is given, which makes an analysis possible in 25 minutes.

During the past few years a number of antioxidants have been proposed for use in paper for food packaging. Among these are gum guaiac, resorcinol, lecithin, soya bean flour, and oat flour. Bentz (1) has now reported the use of butylated hydroxyanisole in paper. Before any chemical can be accepted for use in paper in contact with foods however, accurate methods of analysis must be available.

[1] Sales representative for Tennessee Eastman Company, Division of Eastman Kodak Company.

The use of 2,2′-bipyridine and ferric chloride has been widely accepted (5) as entirely satisfactory for the analysis of butylated hydroxyanisole in fats and oils when other phenolic antioxidants are not present. Since this method was already available (2), the only problem involved in the analysis of BHA in paper and paperboard was to remove the BHA from these materials and get it into an alcoholic solution.

Quantitative Analysis of BHA in Paper

Long Method

When BHA is applied to paper and paperboard by the usual techniques, it is dispersed in extremely fine particles over a vast surface area, which means that any method of removing BHA from these materials must involve intimate contact of a suitable solvent for BHA with all the fibers of the paper and paperboard. It appeared that a soxhlet extraction would