ols whose size did not exceed an average of 9.5 allyl alcohol units.

3. Polyallyl alcohol proved to be an excellent oil upgrader, the mixed glyceryl polyallyl esters of soybean oil at a 10-15% polyallyl alcohol level were superior to linseed oil in film properties. Complete soybean or linseed esters containing 21-25% of polyallyl alcohol were found to be superior to standard soybean and linseed varnish oils in 30-gal. pentaerythritol ester gum varnishes and equivalent to dehydrated castor in short length maleic resin varnishes.

4. A soybean fatty acid-rosin polyester prepared from 47.1% of fatty acids, 31.4% polymerized rosin and 21.5% of polyallyl alcohol X-101 was equivalent to a soybean oil copolymer or a medium oil length castor alkyd as a baking enamel vehicle.

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The Pigmentation of "Red" Cottonseed Oils

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THE price obtainable by the cottonseed processor for his oil is a direct function of the red color of that oil when refined, since he is required to market his oil on the basis of the red color of the refined product (4). Consequently the processor is necessarily concerned with the problem of controlling the development of red color in the crude oil or reducing it to a minimum by refining. In most cases little difficulty is encountered in reducing the red color of a crude oil to an acceptable level by standard refining and bleaching techniques. Occasionally, however, the removal of the red color of certain oils does present a serious problem. For example, oils produced from seed grown in certain areas of West Texas and oils processed under slightly abnormal conditions which tend to develop high red color on storage ("reverting" oils) are very difficult to refine and bleach to a low red color. Despite the interest of the cottonseed industry in "red" color there is little information available concerning the pigments in the oil which are responsible for such color development.

This investigation was initiated in an effort to obtain some information concerning the pigments responsible for red color in cottonseed oils and to attempt to render a typical highly-colored crude cottonseed oil more amenable to standard refining and bleaching techniques by converting the pigments responsible for red color to forms readily removable with alkali.

The unusually high red color of the screw-pressed cottonseed oil produced in a mill in West Texas during the 1950-51 processing season provided an opportunity for such an investigation. To obtain an oil for comparison, the corresponding seed from which this screw-pressed oil had been processed was expressed in a pilot-plant hydraulic press without the application of heat. The screw-pressed oil and its corresponding seed were shipped by air mail immediately after the oil was collected from the presses so that the samples were less than 24 hours old when this investigation was begun. Both the screw-pressed oil and the cold-

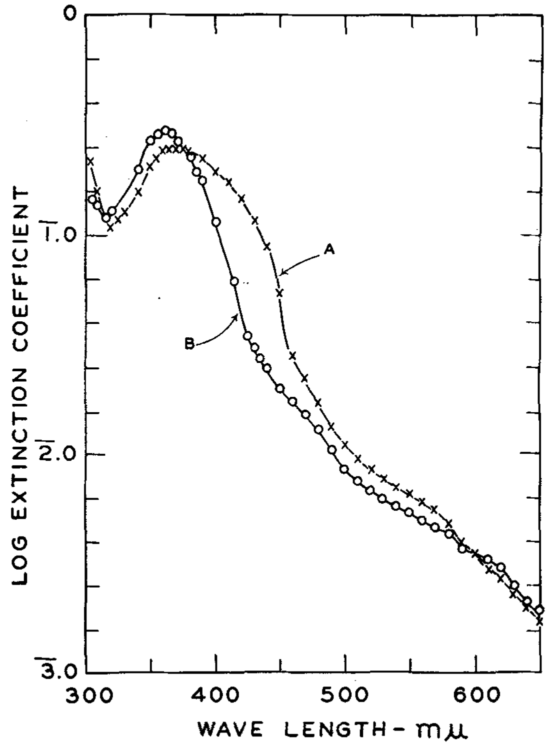

FIG. 1. Absorption spectra of the crude screw-pressed cottonseed oil in iso-octane solution (A) untreated and (b) after treatment with 0.5 *N* HCl.

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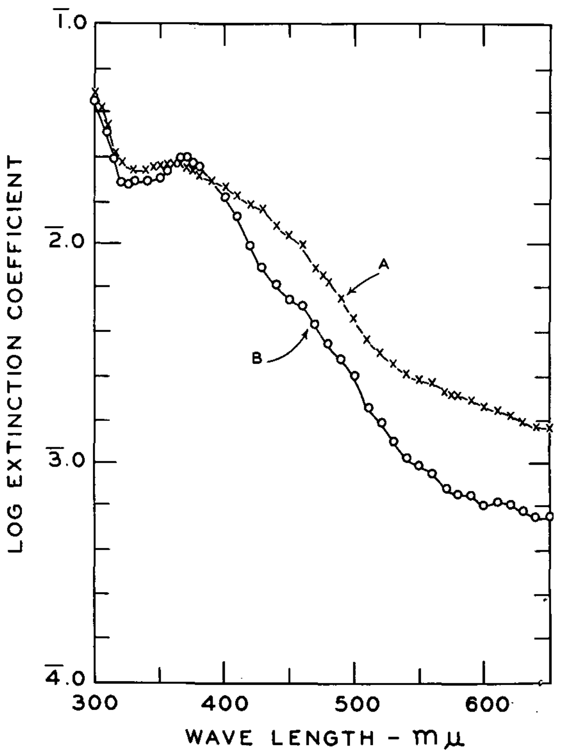

Fig. 2. Absorption spectra of the pilot-plant cold-pressed cottonseed oil in chloroform solution (A) untreated and (B) after treatment with 0.5 N HCl.

pressed oil were stored during the course of this work in a refrigerated room at about 38° F.

Before attempting any treatment of the crude screw-pressed oil, its chloroform solution, as well as that of the cold-pressed oil, was examined spectrophotometrically for the presence of the purple cottonseed pigment, gossypurpurin, since previous investigators had indicated that this pigment or its decomposition products may be responsible for the red color of cottonseed oils (1, 2). The absorption spectra of the crude commercial oil showed little evidence for the presence of gossypurpurin whereas that of the cold-pressed oil gave some evidence of its presence. (Figures 1 and 2) It was believed that, in the former case, the absorption due to gossypurpurin was masked by the presence of other pigments, not present in the cold-pressed oil, which absorbed in adjacent wavelength regions.

Since gossypurpurin is converted to gossypol by treatment with acid (7), and since gossypol is alkali-soluble owing to its polyphenolic nature, the dark-colored screw-pressed oil was treated with acid prior to alkali-refining in an attempt to produce a refined oil which would bleach to a low red color. Both the crude screw-pressed oil and the crude cold-pressed oil were treated with hydrochloric acid (0.5 N), with stirring, at the temperature of the steam bath. The refined and bleach colors of the resulting oils were lower than those of the untreated oils; the effect obviously was more apparent in the case of the dark screw-pressed oil. However this treatment did not result in complete conversion of all the pigments responsible for red color to gossypol or some other alkali-removable form.

Examination of the absorption spectra of the crude screw-pressed oil after acid treatment showed that the characteristic absorption of the original crude oil was shifted from 370-71 mμ towards the region in which the characteristic absorption of gossypol occurs (360-370 mμ). There was however very little change in the absorption at 550 mμ. (Figure 1)

In the absorption spectra of the cold-pressed oil, the effect of acid treatment on the gossypurpurin and/or other gossypol-like pigments present was more pronounced, probably because smaller amounts of the other pigments responsible for the red color in screw-pressed oils are produced by cold-pressing. Acid treatment produced a definite reduction in the spectral absorption in the region of 550 mμ with no apparent change in the region of characteristic absorption of gossypol (360-370 mμ). (Figure 2)

Refined Oils. The oils obtained by refining and bleaching from the acid-treated commercial screw-pressed oil and the cold-pressed oil according to the official methods of the American Oil Chemists' Society (5) were compared to those refined and bleached oils obtained from the untreated crude oils.

In the case of the commercial oil no reduction could be seen in the absorption in either the gossypol region or the red region of the spectra of the refined oils as a result of acid treatment. The spectral absorption of the bleached oil from the acid-treated crude oil showed a greater ratio of absorption at 670 mμ to that at 550 mμ than did the corresponding untreated oil. (The spectral absorption in the region of 670 mμ of oils from West Texas is usually considerably higher than in other screw-pressed and hydraulic-pressed oils.)

The Lovibond red colors of the refined and bleached oils were appreciably lower for the acid-treated oil (refined—35 yellow, 9.2 red; bleached—35 yellow, 4.4 red) than the untreated (refined—35 yellow, 12.7 red; bleached—35 yellow, 7.9 red), but in neither case was the oil prime with respect to color.

As previously stated, the refined and bleach colors of the untreated cold-pressed oil were very low; there-

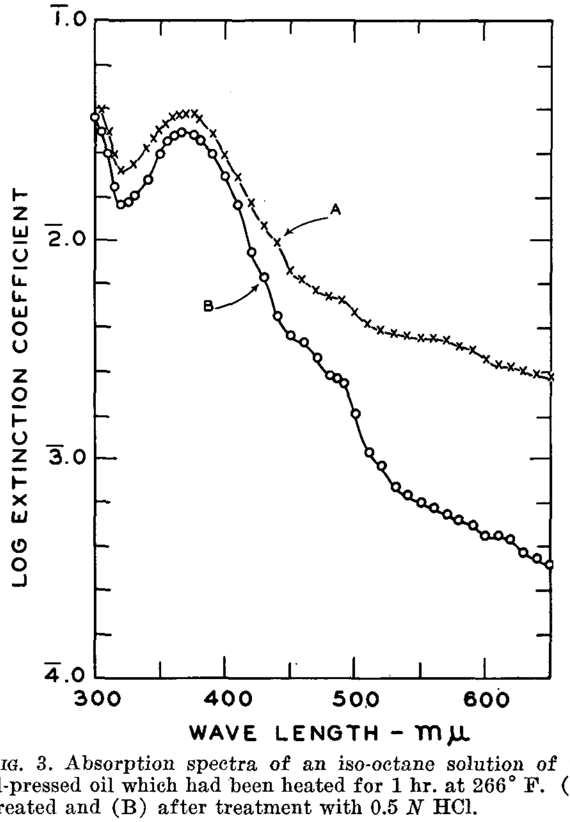

Fig. 3. Absorption spectra of an iso-octane solution of the cold-pressed oil which had been heated for 1 hr. at 266° F. (A) untreated and (B) after treatment with 0.5 N HCl.

fore, as was anticipated, no significant decrease in refined and bleach colors as a result of acid treatment was encountered.

Influence of Heat on Cold-Pressed Oil. A study was made of the effect of heat upon the development of red pigments in the cold-pressed oil, which would not be readily removable by conventional refining and bleaching. The original crude cold-pressed oil, which had refined and bleached very well, even without acid treatment, was heated for one hour at the highest temperature (266° F.) usually attained during the processing of cottonseed by the screw-press method. Significant changes were noted in the absorption spectra as a result of this treatment. (Curves A, Figures 2 and 3). There was a slight decrease in the absorption in the red region (550 mμ) as well as a decrease in the region of maximum gossypol absorption (360-370 mμ) with a broadening of the maximum at this point.

The Lovibond red colors of the refined (70 yellow and 31.7 red) and bleached (35 yellow and 8.3 red) oils from the heated cold-pressed oil were very high compared to those of the original refined and bleached oils (refined—35 yellow and 2.7 red; bleached—10 yellow and 0.7 red).

Acid treatment of the heated cold-pressed oils prior to refining produced some conversion of the red pigments to alkali-removable forms, but, even so, complete conversion or removal was not effected. When a comparison was made of the absorption spectra of the crude, heated, cold-pressed oil and that of the same oil after acid treatment, it was noted that the absorption in the 550 mμ region decreased and the band due to gossypol sharpened as a result of acid treatment. (Figure 3) The Lovibond colors of the refined oils from this acid-treated, heated cold-pressed oil (refined—35 yellow, 20.5 red; bleached—35 yellow, 4.4 red), were lower than those from the heated cold-pressed oil but not as low as the original cold-pressed oil (see above).

Although the investigations reported above offer no practical solution to the problem of reducing red color in highly-colored cottonseed oils, they have served to point out some new facts concerning the nature of the pigments responsible for this red color which might be of help in future investigations of the color problems of "red" cottonseed oils. These facts may be outlined briefly as follows:

1. The change in pigmentation produced by heating cold-pressed cottonseed oil is at least one of those occurring in the oil produced by the commercial screw-press method.
2. Acid treatment of the heated cold-pressed oil and the commercial screw-pressed oil converts at least some of the pigments causing red color to gossypol or other pigments readily removable by alkali-refining and bleaching.
3. However, even in heated cold-pressed oil, there are some pigments contributing to red color which are not converted by acid treatment to readily removable forms. The amount of such pigment is much greater in the screw-pressed oil than in the heated cold-pressed oil. This effect is probably due to the more complex changes in pigmentation which necessarily occur during cooking of the seed and expression of the oil under the greater pressures and higher temperatures involved in the screw-press operation.

Precipitation of an Anthocyanin During Acid Treatment. In the course of experimenting with acid treatment of the West Texas commercial screw-pressed oil prior to alkali-refining, a dark red pigment resembling an anthocyanin was isolated. It was obtained by methanolic hydrochloric acid extraction of a red substance which separated between the aqueous and oily layers during treatment with dilute acid.

It had been suggested previously that aqueous extracts of the cottonseed contain an anthocyanin pigment (3, 6), but the isolation of such a pigment from cottonseed oil has never been reported.

The purification of the crude pigment was very difficult owing to co-precipitation of other materials during acid treatment. Because of this difficulty, specific identification of the pigment could not be made. The purest sample which was obtained melted at 195-197° C. and contained 68.77% carbon, 10.10% hydrogen, and 1.68% ash. The carbon and hydrogen values were higher than those for most of the known glucosidic anthocyanins. However it is possible that the pigment exists in the oil as a glucoside of a disaccharide or polysaccharide or as an ester of a higher fatty acid. The pigment contained no chlorine and therefore could not be a hydrochloride of an anthocyanin. Any phosphatides that may have separated as a result of the acid treatment must have been removed during the purification as the final product contained no phosphorus.

The pigment exhibited the characteristic color change of anthocyanins with change in p*H*, i.e., it was red in the presence of acid and green in the presence of alkali. On standing, the pigment became colorless in alkaline solution, which is typical of anthocyanins. It also gave the typical violet color when subjected to the hydrogen peroxide test. The absorption spectra of a solution of the red pigment in a 2% solution of methanolic hydrochloric acid exhibited maxima at 360-365 mμ and 545-550 mμ. (Figure 4)

By a Soxhlet extraction of the original red precipitate, prior to purification, with hot ethyl acetate, a white crystalline material, melting at 215-217° C. and containing 71.76% carbon, 10.39% hydrogen, and 0.47% ash, was isolated. Because of its solubility in

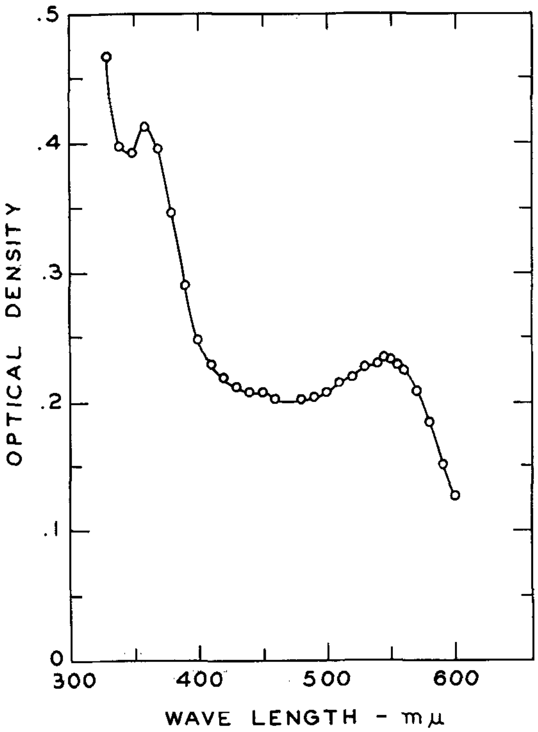

FIG. 4. Absorption spectra of the red pigment in a 2% solution of hydrochloric acid in methanol.

hot ethyl acetate, this product was thought to be either a leucoanthocyanin or the colorless anthocyanin pseudobase.

No red pigment was obtained when the cold-pressed oil employed in these studies was acid-treated.

It was considered important to check whether the presence of an anthocyanin pigment which would separate out on acid treatment was peculiar to the particular screw-pressed oil under investigation or whether other dark oils from different seed or processed under different conditions would contain similar pigmentation. Therefore two more darkly-colored screw-pressed oils, from another mill in West Texas and from a South Texas mill, and three darkly-colored commercially hydraulic-pressed oils from different mills were treated with acid in the same manner. The same red precipitate was obtained in the case of the other two screw-pressed oils but in smaller amount than the original West Texas oil. No red precipitate was obtained from any of the hydraulic-pressed oils tested. The indication implied by the results of these trials of acid-treating highly-colored oils of different histories is that the conditions of the screw-pressing operation alone are favorable to the formation of anthocyanins in the oil or to the extrusion of such pigments from the flakes during pressing. Therefore it seems highly possible that anthocyanins, their precursors, or their decomposition products contribute greatly to the dark color of "red" screw-pressed cottonseed oils which are difficult to refine and bleach.

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Binary Freezing-Point Diagrams for Alpha- and Beta-Eleostearic Acids with Each Other and with Acetamide

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BINARY freezing-point diagrams of adjacent pairs of even-numbered members of a homologous series of long-chain saturated fatty acids indicated that 1:1 molecular compounds are formed (7). However binary systems of fatty acids with one or both components unsaturated seem always to be of the simple eutectic type (7). It has previously been demonstrated by freezing-point data that acetamide forms such equimolecular compounds not only with long-chain saturated fatty acids (6) but also with monounsaturated fatty acids (8). The present report deals with the freezing-point diagrams for the binary systems between two conjugated triply-unsaturated fatty acids, alpha- and beta-eleostearic acids, and between each of these acids and acetamide.

Preparation of Materials

Alpha-eleostearic acid. About 200 g. of fresh tung oil was saponified in a 4-l. flask containing 1,200 ml. of 8% KOH in ethanol by heating at 70-80°C. until 10 minutes after the solution became clear. The solution was cooled to 50°C., diluted with 2,000 ml. of distilled water, and acidified with conc. HCl. The aqueous layer was drawn off with vacuum, leaving the crude acids, which were washed three times with 500-ml. portions of distilled water. The crude acids were then dissolved in 2,000 ml. of Skellysolve B,[2] transferred to a separatory funnel for the removal of the water layer, and the solution filtered through absorbent cotton. After cooling the solution to —30°C. the precipitated acids were filtered off and redissolved in 400-500 ml. of acetone. The acetone solution was slowly cooled, and the first 10-15 g. of acids which precipitated were filtered off and discarded. The solution was cooled to 0°, held there for 1-2 hrs. and the precipitate filtered off. This second precipitate was recrystallized twice more from acetone, using just enough acetone to dissolve the acids at 25°C. and then removing the precipitate formed on cooling the solution to 0°C. The precipitate obtained from the third recrystallization was dried for 20 minutes under vacuum and then dissolved in enough Skellysolve F to give a 10% solution, which was filtered at room temperature with vacuum through a Buchner funnel. This filtrate was cooled to —30°C., and the crystals were filtered off and dried under vacuum at room temperature. Throughout all of the above operations solutions were kept blanketed with nitrogen, and filtrations were carried out rapidly to minimize exposure to air. The product, about 50 g., was stored in

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[2] Mention of trade products does not imply that they are endorsed or recommended by the Department of Agriculture over similar products not mentioned.