Processing of Cottonseed.

II. Factors Determining the Distribution and Properties of Pigments in Products Prepared by Solvent Extraction[1]

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ALTHOUGH direct solvent extraction has been recommended for many years (1-7) as a method for processing cottonseed for oil, it has only recently been applied on an industrial scale in the United States and does not appear to be widely practised in other countries. The successful application of this method for processing soybean (8), as well as current trends toward the development of continuous processes requiring a minimum use of labor, have stimulated interest in the direct solvent extraction of cottonseed.

For many years meals obtained by forepressing oilseeds in hydraulic and continuous screw presses have been extracted with solvents to recover their residual oil. Cottonseed oils produced in this manner are reported (6, 7) to be less highly pigmented than the forepressed oils. Olcott (9) has recommended cooking of cottonseed prior to solvent extraction in order to obtain meals suitable for livestock feed. He reported that oil extracted with petroleum naphtha from precooked seed is comparable to expressed oils with respect to refining loss and color. However, since precooking of cottonseed has been reported (9) not to increase the yield of extracted oil, it would appear to be a superfluous operation. Cooking of cottonseed also denatures the protein, partially or completely destroys other thermally unstable, nutritive constituents of the seed, and darkens the meal so that it is unsuitable as a source of protein for industrial use. Consequently, with the development of methods for the elimination or control of color in the products, direct solvent extraction of uncooked cottonseed should offer many advantages.

Control of color is not the only problem which is encountered in the successful processing of cottonseed by direct solvent extraction, but it is an important one. The unique system of pigments in the cottonseed kernel not only differentiates it from other commercial oilseeds, but it is also responsible for many of the difficulties encountered in adapting the usual solvent extraction procedures to the processing of this seed. Recent publications (6, 7, 9, 10, 11) which have reviewed the advantages and disadvantages of solvent extraction of cottonseed have been concerned principally with the difficulties involved in avoiding or removing color in the extracted oil, and with the destruction or removal of pigments of the meal.

Pigments of Cottonseed

The pigment content of cottonseed is unusually high and variable. Gossypol, the principal pigment of cottonseed, has been found (12) to occur in concentrations as high as 2% of the weight of the kernel. Gossypurpurin (12, 13, 14) has been found to constitute as much as 0.055% of the weight of the kernel. Gossyfulvin (15, 16) has been detected in low concentrations in only a few samples of cottonseed which had been stored at high moisture content. The concentration of the yellow, oil-soluble carotenoid in cottonseed has been reported (17, 18) to vary from 0.096 to 0.219% of the weight of the kernel. Another yellow, oil-soluble pigment has recently been detected in cottonseed (12), but it has not yet been isolated in a pure state so that the constants necessary for determining its concentration in the seed have not been established. Gossycaerulin (19) has been detected only in cooked cottonseed.

The gossypol content of different samples of cottonseed may vary many-fold and has been shown (20, 21, 22, 23, 24) to be dependent upon a number of factors, including species, variety, location and year of growth, maturity, and length and conditions of storage of the seed. The concentration of cottonseed pigments other than gossypol in the seed has not yet been investigated exhaustively, but their variation seems to be of approximately the same order as that of gossypol and to be dependent upon as many factors.

Most of the complex polyphenolic pigments of cottonseed readily undergo alteration. Gossypol is so unstable after its isolation from other seed components that simple derivatives can be prepared only under mild and carefully controlled reaction conditions. Solutions of gossypol are stable for only a very short time even in relatively inert solvents. The other related pigments of cottonseed, gossypurpurin, and gossyfulvin are even more unstable than gossypol whereas the carotenoid pigment and the yellow extraglandular pigment are relatively stable.

The yellow, oil-soluble pigment has been shown (12) to occur in solution in the oil in the extraglandular tissue of the cottonseed. It also appears probable (12) that the carotenoid pigment occurs in the oil in the extraglandular tissue. On the other hand, all of the gossypol and gossypurpurin of the cottonseed kernel has been shown (12) to be segregated in the pigment glands. Since the last mentioned pigments are the most abundant, the most deeply colored, and the most unstable of the pigments of the kernel, most of the dark color observed in solvent-extracted oil and meal can be attributed to them or their decomposition products.

Behavior of the Pigment Glands

The earliest investigators (25, 26, 27, 28, 29) of the anatomy of the cottonseed noted the occurrence of the pigment glands and reported observations of their

[1] Presented in part at a symposium on Solvent Extraction of Cottonseed at the 37th Annual Meeting of the American Oil Chemists' Society, New Orleans, Louisiana, May 15-17, 1946.

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reaction with water. Carruth (30) suggested the probable importance of these glands in the transformation which the pigments undergo during processing of cottonseed. Nevertheless, for many years the role of the pigment glands was overlooked, but the properties of the pigment gland have recently been shown (12, 14, 22, 31, 32) to determine to a considerable degree the behavior of the pigments during processing of cottonseed by either expression or solvent extraction methods.

The pigment glands, which contain all of the gossypol and gossypurpurin of the seed, possess a thick, strong, resistant wall which presumably protects the gland contents from direct contact with the components of the surrounding tissue in the intact seed. These glands possess such high mechanical strength that in seed of normal moisture content only a small fraction of them are ruptured under the pressures and shearing stresses to which they are subjected during rolling or grinding of seed preparatory to pressing or extraction. Consequently, the pigment glands containing the intraglandular pigments remain in the meal unless processing conditions are such as to rupture the gland walls.

The gland walls have been shown (12, 31, 32) to be resistant to the action of most liquids except water and a few water-miscible organic liquids of low molecular weight. Contact with water produces instantaneous rupture of the walls and the expulsion of the gland contents. The sensitivity of the glands to moisture is so great that they are affected by traces of moisture and their sensitivity increases as the temperature is increased (14).

Methanol, ethanol, isopropanol, acetone, and 1,4-dioxane have been shown (12, 31, 32) to rupture the pigment gland wall. Although the action of these solvents is fairly rapid, it is slower than that of water, and the speed with which aqueous mixtures of these solvents attack the pigment glands is proportional to the amount of water in the mixture. The pigment glands are ruptured most rapidly by mixtures containing such high proportions of water that the pigments are expelled from the glands in streams of suspended particles. Addition of more of the organic solvent accomplishes solution of the suspended pigments. Thus, rapid extraction of both the oil and the pigments is most efficiently obtained by first treating the seed with water or with a dilute aqueous solution of the organic solvent, and then adding organic solvent in sufficient quantity to dissolve the pigments and oil.

Organic liquids other than the aforementioned water-miscible solvents have been found (12, 31, 32) to fall into two categories with respect to their activity toward the glands: (a) those which completely extract the gossypol from the glands after contact for 24 hours or less, and (b) those having little or no effect on the glands even after prolonged contact. The *active* group includes diethyl ether, chloroform, and probably other chlorinated hydrocarbons. The slow extraction of the intraglandular pigments effected by these solvents was shown (12, 32) to be the result of their normal, slight content of moisture. Thus, the *active* group differs from the *inert* group only by the affinity of the solvents of the former group for water, and their consequent tendency to contain varying amounts of moisture. In the presence of moisture, hydrocarbons which normally do not affect the pigment glands were found (12) to extract appreciable amounts of the intraglandular pigments.

The amount of pigments extracted by organic solvents other than the water-miscible group was found (12) to be determined not only by moisture during extraction, but also by the original moisture content of the seed kernels when they were rolled or ground preparatory to extraction.

Solubility of Cottonseed Pigments in Mixtures of Oil and Solvent

The carotenoid pigment of cottonseed has been reported (17, 18) to be soluble in cottonseed oil, as well as in both polar and non-polar organic solvents such as petroleum naphthas and aqueous ethanol. The recently detected, non-acidic, yellow, extraglandular pigment of cottonseed (12) is soluble in cottonseed oil and in extracts of the oil obtained with a large number of organic solvents. Consequently, all solvent-extracted cottonseed oils will contain all of these two pigments which are present in the original seed.

Gossypol is very soluble in cottonseed oil, acetone, and 1,4-dioxane. Although it is of limited solubility in diethyl ether, benzene, chlorinated hydrocarbons, methanol, ethanol, and isopropanol, most of the gossypol from ruptured glands is dissolved in the relatively large volumes of these solvents which are normally employed for extraction of the oil.

Gossypol is very slightly soluble in petroleum naphthas but is more soluble in the higher than in the lower boiling hydrocarbons. Because of the solvent action of cottonseed oil for gossypol, the ratio of solvent to seed during the initial stages of extraction with petroleum naphtha will largely determine the amount of gossypol extracted from seed of a given content of moisture. Usually, however, petroleum naphtha does not extract all of the gossypol from the small number of glands ruptured under normal conditions, and, in the case of seed which has been wetted in order to rupture all of the pigment glands, light petroleum naphtha was found (12) to extract only about half of the total gossypol present in the seed.

Gossypurpurin (13) is insoluble in cottonseed oil and in most of the usual organic solvents, except acetone and 1,4-dioxane. It is slightly soluble in diethyl ether and chloroform, and its solubility appears to be enhanced by the presence of preponderant amounts of gossypol. Consequently, these solvents, as well as acetone and 1,4-dioxane, will usually extract all of the gossypurpurin from ruptured pigment glands. Because of the very limited solubility of gossypurpurin in methanol, ethanol, and isopropanol, these solvents extract all of the gossypurpurin only from seed containing very small amounts of this pigment. Since gossypurpurin is very unstable in all organic solvents except chloroform at relatively low temperatures, extracts of cottonseed usually contain gossypurpurin in the form of its yellow decomposition product.

Pigments of the Hulls

The characteristic red-brown color of cottonseed hulls has been reported (33) to be largely attributable to the presence of a stable xylan, which is extracted only by 2% alcoholic sodium hydroxide (33) and to some extent by water (34). The isolated pigment of the hull is insoluble in cold absolute ethanol, diethyl ether, and acetone (33).

The presence of hulls was found to have no effect on the color of oils extracted with liquid propane or butane (35), commercial hexane (36), or chloroform (14). Shrader (3) attributed the dark color of the oil which he obtained by extraction of cottonseed with benzene to the presence of hulls. However, in view of the failure of other hydrocarbon solvents to extract any color from hulls, the color of the oil obtained by Shrader was probably attributable to pigments of the kernels rather than of the hulls.

A very small amount of gossypol is extracted from cottonseed hulls by 60% aqueous ethanol, but the amount is so small that it is probably attributable to contamination of the hulls with fragments of kernels.

On the basis of these observations it can be concluded that the pigments of cottonseed hulls are unaffected during extraction of the oil with the usual organic solvents and that they remain in the hulls which are admixed with the extracted meal.

Pigment Content of Solvent-extracted Oils

Oils obtained by extraction of cottonseed with acetone or 1,4-dioxane have been found (12) to contain all of the extraglandular pigments and all of the gossypol originally present in the seed, and the yellow decomposition product of gossypurpurin. Methanol, ethanol, isopropanol, or aqueous mixtures of these solvents also extract all of the cottonseed pigments except in the case of seed containing relatively large amounts of gossypurpurin.

When precautions are taken to exclude all moisture, organic solvents other than the aforementioned were found (12) to extract only the extraglandular oil-soluble pigments. On the basis of the frequently reported observation (9, 10, 11, 12, 22, 35, 36, 37) that commercial grade petroleum naphthas and liquid hydrocarbons of low molecular weight produce very lightly colored oil, it can be concluded that these solvents normally contain little moisture.

Unless precautions are taken frequently or continuously to remove moisture from the system it will accumulate during large scale continuous extraction of cottonseed and result in increased rupture of the pigment glands with consequent increase in the color of the extracted oil. Thus, Goldovski and Podolskaya (39, 40) have reported that cottonseed oil obtained by extraction of seed of unknown moisture content with petroleum naphtha (benzine) contained 10 to 15% of the gossypol of the original seed when continuous extractions were carried out on an industrial scale at 70° to 80° C. for periods as long as thirty hours.

Increase in temperature has also been found (14) to increase the tendency of the pigment glands to rupture in the presence of moisture. Rosenthal (35) observed that oils extracted with liquid propane and butane at very low temperatures are very much less colored than are oils extracted with the same solvents at higher temperatures. These differences may be attributed to the increased number of pigment glands ruptured during extraction at the higher temperatures.

Reports (9, 10, 37) that extraction of cottonseed with chlorinated hydrocarbon solvents usually produce deeply colored oils and conflicting reports (2, 3, 4, 5) relative to the color of cottonseed oil produced by extraction with benzene cannot be satisfactorily explained on the basis of published data. The dark color of the oils extracted with the solvents may have resulted from either moisture, or elevated temperatures, or both.

The pigmentation of cottonseed oil obtained by extraction of the seed with organic solvents, other than the water-miscible organic liquids which are capable of rupturing the pigment glands in the absence of moisture, can be summarized as follows: Under normal operating conditions, in the presence of moderate amounts of moisture, and when elevated temperatures are avoided, the amount of the intraglandular pigments extracted with the oil is very slight. The amount of pigments which will be extracted by a specific solvent is proportional to the amount of moisture in the seed and in the solvent, the length of the extraction period, the temperature during extraction, and the relative solubilities of the different cottonseed pigments in the solvent.

Refining of Solvent-extracted Cottonseed Oil

Sievers and McIntyre (2) investigated the refining characteristics of cottonseed oils extracted at room temperature with diethyl ether, benzene, carbon tetrachloride, trichlorethylene, *light* gasoline, and *heavy* gasoline. All of the crude oils were reported to be deeply colored and could be refined to an acceptable color only with the use of a large excess of alkali. The crude oils which Shrader (3) obtained by extraction of cottonseed with benzene were found to require treatment with excess alkali and re-refining in order to remove objectionable color. Wesson (4, 5) reported, however, that a small scale pilot-plant extraction of cottonseed with benzene yielded an oil which was readily refined to a *good* color.

The deeply colored oils obtained by extraction with chlorinated hydrocarbons have been reported (37) to yield lightly colored refined oils when the miscella was treated with dilute or concentrated alkali before removal of the solvent.

According to Rosenthal (35), cottonseed oil produced by extraction with liquid propane or butane at low temperatures can be refined to yield prime colored oil by the usual alkali treatment without the use of bleaching clay. Oils extracted from seed with the same solvents at higher temperatures and desolventized at moderate temperatures produced prime colored oil when refined by the methods used for expressed oils. Vix, Pollard, Spadaro, and Gastrock (36) have reported that oils extracted with commercial hexane at 70° F. from prime seed were readily refined when the solvent was removed at temperatures not exceeding 110° F. or at temperatures of 150°-180° F. for a very short time.

Because of the aforementioned contradictory reports, a comparison was made of the behavior of extracted oils containing principally the extraglandular pigments with those containing essentially all of the pigments of the kernel. This was accomplished by extracting two samples of the same lot of flaked prime cottonseed in a Soxhlet type apparatus, one with light petroleum naphtha [3] and the other with diethyl ether. Normal operating conditions were simulated by using seed of normal moisture content, 8.44%, and commercial grade solvents. The kernels were found to be very heterogeneous with respect to their gossypol content but contained an average of 1.18% of this pigment.

[3] Commercial pentane-hexane, boiling range 95°-138° F.

Extraction with diethyl ether was continued until all but traces of the pigments had been removed from the residual meal. The extraction was carried out by first refluxing on a steam bath for three hours after which the seed was allowed to remain in contact with ether at room temperature overnight, and the gossypol finally flushed from the seed by refluxing the ether through the extractor for an additional three hours.

Extraction of cottonseed with petroleum naphtha was carried out continuously on a steam bath for a period of six hours, which was the minimum time found to be necessary for complete removal of the oil. After evaporating as much of the solvent as possible on a steam bath, the last traces were removed under vacuum at room temperature. Refining of the solvent-extracted oils was carried out by a modification (41) of the American Oil Chemists' Society official refining method as follows. A 100-g. sample of oil was weighed into a centrifuge bottle and the required amount of lye was added. After shaking, the bottle was placed in a water bath at 65° C. and maintained at this temperature until the foots began to separate which generally occurred in three to seven minutes. The sample was then centrifuged for 20 to 25 minutes, after which the oil was decanted, usually through a funnel fitted with a cotton plug. The refining loss was calculated from the weights of original oil and soapstock in the usual manner. The alkali-refined oil was bleached according to the American Oil Chemists' Society official method and the color of the bleached oil measured in terms of Lovibond red and yellow units.

The crude oil obtained by extraction with diethyl ether was colored almost black, and was very much darker than that obtained by extraction with petroleum naphtha. However, as may be seen from the data in Table 1, the diethyl ether extracted oil was very much lighter after bleaching than the corresponding bleached petroleum naphtha-extracted oil.

TABLE 1.

Refining Characteristics[a] of Diethyl Ether- and Light Petroleum Naphtha-Extracted Cottonseed Oil.

Solvent	Free fatty acid %	Lye[b] Be°	Color of oil (Lovibond)				Depth of cell, inches
			Refined oil		Bleached oil[c]		
			Y	R	Y	R	
Diethyl ether.....	3.5	20	35	25.5	35	1.4	5¼
Petroleum naphtha..	0.34	20	35	7.5	35	5.2	1

[a] Refining of the oils was carried out according to a small-scale modification of the Official American Oil Chemists' Society method.

[b] Amounts calculated on basis of that required for screw-pressed oils of corresponding FFA according to official methods of the American Oil Chemists' Society.

[c] Bleached with 6% American Oil Chemists' Society Official Fuller's earth.

Comparison of the absorption spectra (Fig. 1) of chloroform solutions of the two crude oils showed that they contained different amounts of gossypol and extraglandular pigment. By application of the antimony trichloride-spectrophotometric method (12, 14, 38), the residual meal was found to contain only 0.025%, and the crude diethyl ether-extracted oil 2.16% of gossypol. The gossypol in the crude diethyl ether-extracted oil represented only 59% of the total gossypol originally present in the flaked seed, thus indicating that almost half of the gossypol decomposed during extraction with diethyl ether. The absorption spectrum of the crude oil (Fig. 1, Curve A) was very similar to that of pure gossypol (16). However, the value of the specific extinction at 364 to 367 mμ, the region of maximum absorption by pure gossypol, was 10.6 for the crude oil. The specific extinction coefficient at the same wavelengths calculated for the absorption due to gossypol in the extract was 7.6, indicating that the crude diethyl ether-extracted oil

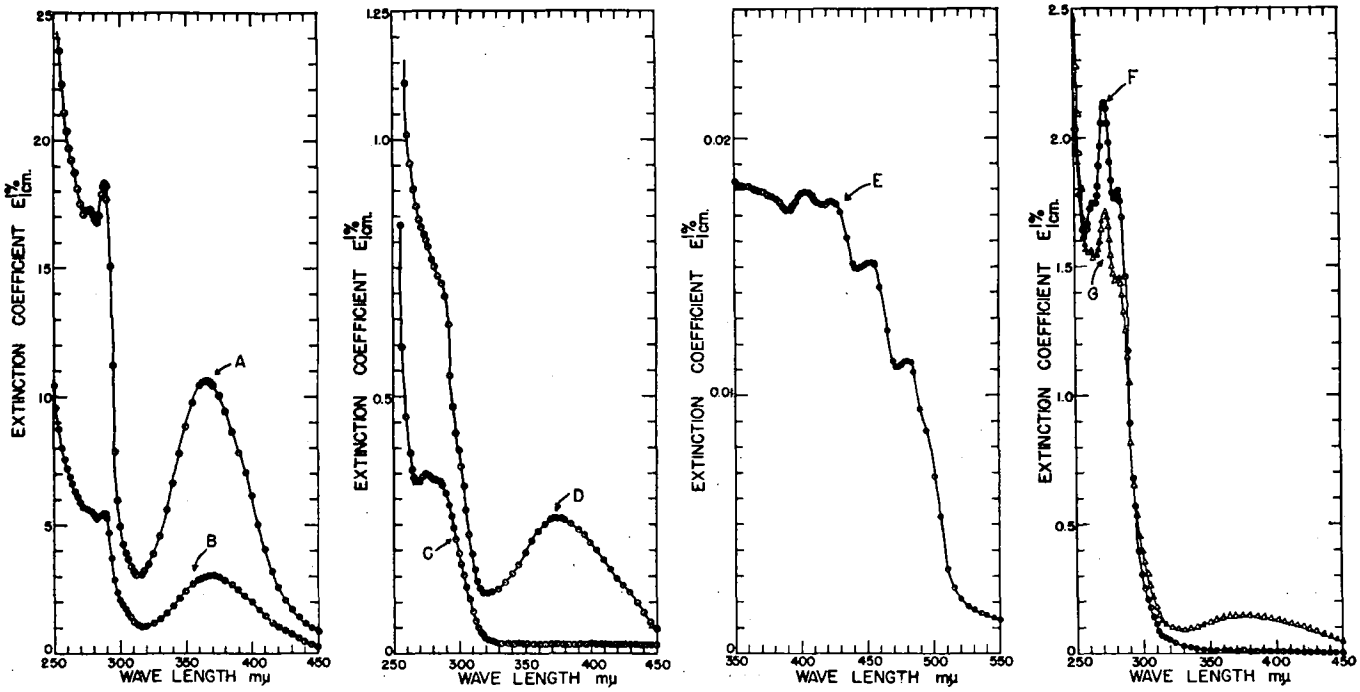

FIG. 1. Absorption spectra of chloroform solutions of (A) diethyl ether-extracted crude cottonseed oil, (B) petroleum naphtha-extracted crude cottonseed oil, (C) and (E) alkali-refined, diethyl ether-extracted cottonseed oil, (D) alkali-refined, petroleum naphtha-extracted cottonseed oil, (F) bleached, diethyl ether-extracted cottonseed oil, and (G) bleached, petroleum naphtha-extracted cottonseed oil.

contained appreciable amounts of some pigment other than gossypol, probably the oil-soluble extraglandular pigment.

The crude oil obtained by extraction of the seed with petroleum naphtha contained only 0.33% gossypol. Its absorption spectrum (Fig. 1, Curve B) indicated the presence of gossypol admixed with appreciable amounts of the oil-soluble extraglandular pigment.

Alkali-refining of the diethyl ether-extracted oil removed all of the gossypol and most of the color, and revealed the presence of pigments which had not previously been detected (Fig. 1, Curves C and E). Since these pigments were completely adsorbed during bleaching (Fig. 1, Curve F), it could be inferred that they were relatively polar compounds.

Alkali-refining of the crude oil obtained by extraction with petroleum naphtha removed all of the gossypol but did not appreciably reduce the color. The absorption spectrum of the alkali-refined oil (Fig. 1, Curve D) resembles that of extracts (12) obtained by extraction of seed with petroleum naphtha in the absence of moisture. It can, therefore, be inferred that only the extraglandular yellow pigment remained in the refined oil. As may be seen from the absorption spectrum (Fig. 1, Curve G) and the Lovibond colors (Table 1), bleaching removed very little of this pigment.

On the basis of these observations, it can be concluded that gossypol is readily removed during alkali-refining of solvent-extracted oils. Moreover, since all of the extraglandular pigment of the original seed must necessarily have been extracted by diethyl ether but is not detectable in the refined or bleached oils, it can be inferred that this pigment is objectionable only in oils obtained by extraction with petroleum naphthas.

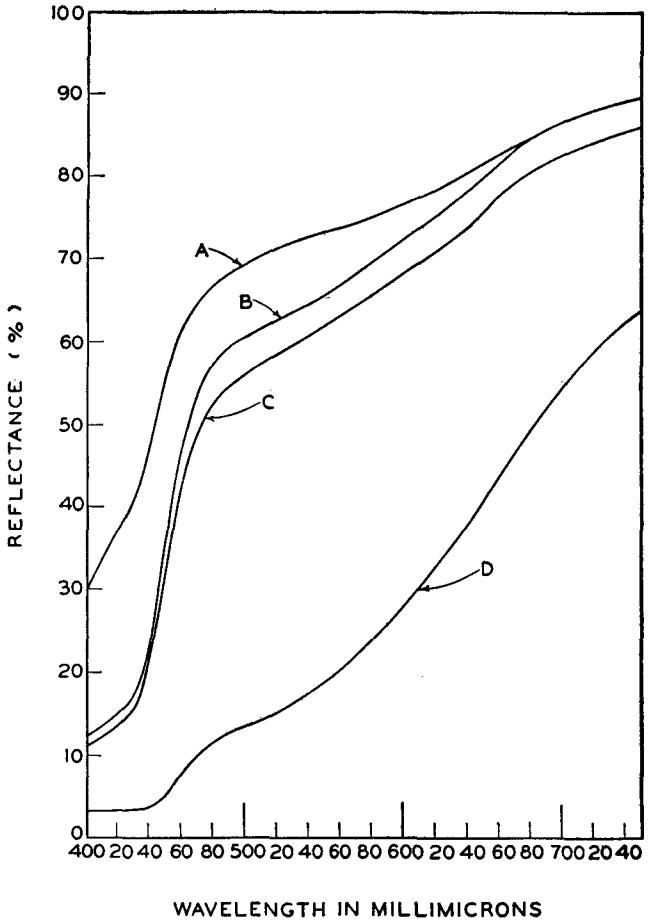

FIG. 2. Reflectance spectra of cottonseed meals, after extraction with (A) 1,4-dioxane, (B) commercial hexane, (C) 95% ethanol, and (D) 60% aqueous ethanol.

Color Fixation in Solvent-extracted Oils

Several investigators (35, 36, 39, 40, 42-46) have noted that exposure of expressed or solvent-extracted oils to elevated temperatures results in darkening of the crude, and the corresponding alkali-refined and bleached oils obtained. Oils produced by extraction with petroleum naphtha or other hydrocarbon solvents have been reported (35, 36, 39, 40, 46) to be particularly susceptible to *color fixation*. The *fixation of color* caused by heating crude petroleum naphtha-extracted oils has been correlated with their content of gossypol (39, 40), and parallel changes in color have been observed upon heating solutions of pure gossypol in cottonseed oil (44, 45) and in benzene (45).

Since all solvent-extracted oils produced under normal processing conditions contain some gossypol, and exposure of the crude oils to heat during extraction and subsequent removal of the solvent cannot be entirely avoided when operations are carried out on a large scale, some *fixation of color* will always occur. Consequently, it is of practical, as well as theoretical importance, to determine the nature of the reaction as an aid in removing so-called *fixed* gossypol from petroleum naphtha-extracted oils.

Podolskaya (44, 45) has reported the only work concerning the nature of the thermally induced decomposition of gossypol which results in *color fixation*. This investigator observed the same sequence of color changes when solutions of gossypol in cottonseed oil were heated in an atmosphere of carbon dioxide or of oxygen and concluded that *color fixation* does not involve oxidation of gossypol.

During the present investigation it was found that gossypol was completely removed during refining of oils obtained by extraction with petroleum naphtha or diethyl ether. The residual high color of refined and bleached oils produced from petroleum naphtha-extracted cottonseed oil is therefore attributable entirely to the stable, oil-soluble pigment which occurs in the extraglandular tissue of the seed, and it is possible that some of the original extracted gossypol may have been converted to this pigment during heating of the extract. However, it is evident that the extraglandular pigment had been converted to more polar and hence more readily removed decomposition products during contact with diethyl ether, since no trace of this pigment could be detected in the diethyl ether-extracted oil after refining and bleaching. It may therefore be concluded that the nature of the reaction medium furnished by the solvent is much more important than the original pigment content of solvent-extracted oils in determining the final colors of the refined and bleached oils. Since oxidative decomposition of all of the cottonseed pigments, such as that which occurs during heating of diethyl-ether-extracted oil, results in the formation of pigments which are readily removed during refining and bleaching, it can be predicted that addition of oxidizing agents to petroleum naphtha-extracted oils

will increase the color of the crude oil but reduce the color of the bleached oils obtained therefrom.

Extracts obtained with water-miscible alcohols, ketones, and ethers have been shown (12) to contain essentially all of the pigments present in the cottonseed, but no systematic investigation appears to have been reported with respect to the refining characteristics of oils obtained by extraction with these solvents. However, since these polar solvents form relatively active media, the pigments probably decompose into compounds more polar than the original pigments of the seed so that little difficulty should be experienced in reducing the color of the extracted oils by the usual refining procedures.

Pigmentation of Solvent-extracted Cottonseed Meals

Solvent-extracted cottonseed meals can be grouped into two principal categories with respect to the distribution and total content of pigments. After exhaustive extraction with solvents which are capable of rupturing the pigment glands, only those pigments which are insoluble in the mixture of oil and the solvent employed will remain with the meal. As shown in Table 2, and Fig. 2, acetone and 1,4-dioxane readily extracted all of the pigments from cottonseed and yielded almost colorless meals.

TABLE 2.

Color of Solvent Extracted Cottonseed Meal.

Solvent used for extraction	Color of meal	Per cent reflectance[a] at			
		400 mμ	440 mμ	480 mμ	600 mμ
Aqueous methanol[b]	Light brown	3.2	3.7	11.6	28.0
Aqueous ethanol[b]	Tan	4.5	5.6	16.7	34.0
95% aqueous ethanol	Straw yellow	11.5	15.0	52.5	68.4
Commercial hexane	Yellow[c]	12.5	16.6	57.3	72.5
Light petroleum naphtha	Pale yellow[c]	13.5	18.0	57.8	72.5
Diethyl ether	Almost white	24.3	38.7	63.5	76.2
Dioxane	White	30.1	41.0	67.0	76.7
Acetone	White	33.7	45.5	72.0	81.8

[a] Measured relative to magnesium oxide, with a General Electric Recording Spectrophotometer on meals ground to pass a U. S. No. 100 sieve; both sample and standard were covered with a curved glass disc.

[b] Treated first with an aqueous mixture containing 30% alcohol (by weight) then more concentrated alcohol mixture added to yield final extract of 60% alcohol; extracted meal finally washed repeatedly with anhydrous alcohol.

[c] Intact pigment glands throughout tissue.

Methanol, ethanol, isopropanol, and aqueous mixtures of these solvents rupture all of the pigment glands within a short period of contact and extract all of the gossypol, but only a small amount of gossypurpurin is extracted because of its limited solubility in these solvents. Consequently, extraction with these solvents was found to yield lightly colored meals only when the original seed contained very little gossypurpurin.

Yellow meals, similar to that produced by extraction with 95% ethanol (Table 2, Curve C in Fig. 2), were obtained by extraction with these solvents of seed which had been stored during warm weather so that their content of gossypurpurin had increased. Extraction with aqueous mixtures of the alcohols removes all of the gossypol and some of the gossypurpurin with the oil, but, as shown in Table 2 and Curve D of Fig. 2, the resultant meal is considerably darkened. The darkening of the meal apparently results from the conversion of colorless into colored compounds during contact with water.

Since both gossypol and gossypurpurin are somewhat soluble in diethyl ether, and the pigment glands are slowly ruptured by moist diethyl ether, commercial grades of this solvent can be employed for extraction of the pigments along with the oil. However, because of the limited solubility of water in diethyl ether and the consequent slowness with which the pigment glands are ruptured, meals of the light color shown in Table 2 are obtained only after prolonged extraction with this solvent. The shape of the reflectance spectrum curve of this meal is similar to that obtained with the dioxane-extracted meal but slightly lower, thus indicating that the former meal was somewhat more deeply colored.

Meals obtained by extraction of cottonseed with petroleum naphthas fall into a second category. They are usually colored yellow or pale tan (Table 2), and all of them have reflectance spectra similar to that of the commercial hexane[4]-extracted meal shown in Curve B of Fig. 2. When excessive amounts of moisture and elevated temperatures are avoided during extraction, most of the pigment glands remain intact in the meal, and the protein tissue is colored pale yellow or tan by adsorption of most of the gossypol and yellow decomposition product of gossypurpurin released from the small number of ruptured glands.

Because of the limited solubility of gossypol and gossypurpurin in the hydrocarbon solvents, only a small fraction of these pigments will be extracted with the oil even though most of the glands are ruptured as in the case of extraction in the presence of considerable moisture and at elevated temperatures. Consequently, cottonseed meals obtained by extraction with hydrocarbon solvents contain most of the original gossypol and gossypurpurin of the seed, and the conditions during extraction determine only whether most of the pigments remain within intact glands or are adsorbed on the protein tissue.

Olcott (9) has suggested supplementary extraction of petroleum naphtha-extracted meal with diethyl ether, or an equivalent solvent, in order to remove the residual pigments. However, Fontaine, Detwiler, and Irving (47) have reported that extraction of cottonseed with petroleum naphtha followed by exhaustive extraction with diethyl ether yields meals of a distinct yellow color in contrast to the almost colorless meals obtained by direct extraction of cottonseed with diethyl ether.

The incomplete extraction of the residual gossypol and gossypurpurin of defatted meal accomplished by chloroform and diethyl ether (38) has been shown (12) to be due to adsorption of the pigments and the inability of the relatively non-polar solvents to elute the adsorbed pigments. Goldovski and Podolskaya (39, 40) have reported that steaming of the meal produced by extraction of the seed with petroleum naphtha converts practically all of the residual gossypol to a form which cannot be extracted with diethyl ether. This transformation of gossypol to so-called *bound* gossypol can undoubtedly be attributed to its release from the pigment glands and subsequent adsorption on the protein tissue.

Treatment of defatted cottonseed with methanol, ethanol, isopropanol, acetone, or 1,4-dioxane has been found (12) to remove both the adsorbed gossypol and that contained in intact glands. The alcohols yield colorless meals only when the gossypurpurin content of the original seed is relatively slight whereas meals obtained by final treatment with acetone or 1,4-dioxane are practically colorless regardless of the pigment

[4] Commercial hexane of boiling range 146°-158° F.

content of the original seed or of the defatted meal.

The gland flotation process (31, 48) is applicable to the removal of intact pigment glands from defatted meal. When the original extraction with hydrocarbon solvents has been carried out in the presence of little moisture and at moderate temperatures so that only a small fraction of the glands are ruptured, very lightly colored meals can be obtained by application of the gland flotation process to the defatted seed.

Mechanical Fractionation of Cottonseed by Gland Flotation Process

The gland flotation process is a modified solvent extraction method by means of which the cottonseed kernel is mechanically separated into pigment glands, gland-free meal, and lightly colored oil. This process is made possible by the occurrence of most of the cottonseed pigments in glands and the rather unusual properties of these glands. The cottonseed pigment glands, in addition to being very resistant to rupture, have a density less than that of the other seed tissue. Since the pigment glands are tougher than any other part of the seed kernel, they can be disengaged from other seed tissue by violent agitation of a suspension of flaked seed in a solvent or mixture of solvents, such as hydrocarbons, chlorinated hydrocarbons, and glycerides which do not rupture the pigment glands. By adjusting the density of the solvent to 1.378 g./cc., the intact pigment glands rise to the surface, the gland-free flour sinks to the bottom, and the oil and oil-soluble extraglandular pigments are dissolved in the solvent. The pigment glands and gland-free flour can then be separated mechanically and the oil recovered from the solvent.

Cottonseed oil obtained by direct application of the gland flotation method is analogous to the oils obtained by the usual extraction method employing similar solvents under corresponding conditions. As in the case of the usual extraction procedures, the extent to which the pigment glands are ruptured and the intraglandular pigments extracted with the oil has been shown (31) to be determined by the amount of moisture in the seed and solvent, and the temperature, and length of exposure of the pigment glands to contact with the solvent. The oil obtained by the flotation process will contain all of the extraglandular pigments and varying amounts of the intraglandular pigments. Refining procedures applicable to the oils obtained by extraction with dry hydrocarbon or chlorinated hydrocarbon solvents will be directly applicable to the oils obtained by the gland flotation method.

By preliminary desiccation of the seed before flaking of the kernels, removal of all moisture from the solvents, and violent agitation of the suspended flakes until all of the glands are detached from other seed tissue, it has been possible to obtain meals essentially free of color. Under practical processing conditions (48) when seed of normal moisture content are flaked and the moisture content of the flakes reduced to about 5%, meals obtained by use of commercial grade solvents are very pale yellow in color. They have been found to contain less than 0.039% of adsorbed gossypol and small amounts of the yellow decomposition product of gossypurpurin. Any residual adsorbed pigments can be removed from the meal by treatment with methanol, ethanol, isopropanol, acetone, or 1,4-dioxane.

The yield of pigment glands obtained by the flotation process varies with the number of pigment glands ruptured during processing, and with the original gland content of the seed. The latter has been found (12) to vary from 2 to 5% of the weight of the kernel in the different samples of cottonseed which have been investigated.

Summary

On the basis of the properties of the pigments and pigment glands of cottonseed, criteria have been established which render it possible to obtain oil and meal in which the pigmentation may be controlled more or less specifically at the will of the processor.

For the extraction of oil free of gossypol and gossypurpurin, moisture is excluded from the system and solvents other than water-miscible alcohols, ketones, and ethers are used. Modified refining procedures have been suggested for processing of the solvent-extracted oils. The solvent-extracted meals will contain most of the pigments of the seed. Their distribution in the meal, in intact piment glands or adsorbed on the extraglandular tissue, will depend upon the amount of moisture in the system, and the temperature and duration of the extraction.

As a second alternative, extraction of oil containing essentially all of the pigments of the seed is accomplished with the use of a number of water-miscible solvents capable of rupturing the pigment glands, or with the use of other organic solvents which rupture the pigment glands because of their normal slight content of moisture. The pigments can be readily removed from these oils by the usual refining methods. The pigment content of the solvent-extracted meals will be dependent upon the original pigment content of the seed, and the solubility of gossypol and gossypurpurin in the solvent employed.

As a third alternative, the gland flotation process can be applied to obtain intact pigment glands, and both oil and meal substantially free of pigments in a single operation.

The choice of methods for removal of residual pigments from solvent-extracted meals will be determined by the amount and distribution of pigments in the meal. The gland flotation process can be applied for removal of intact pigment glands. Supplementary extraction of defatted cottonseed meal with methanol, ethanol, isopropanol, acetone or 1,4-dioxane can be employed for removal of gossypol, but of the aforementioned solvents, only acetone and 1,4-dioxane yield lightly colored meals when the gossypurpurin content of the original seed is relatively high.

Acknowledgment

The authors wish to acknowledge their indebtedness to B. A. Brice and M. L. Swain for the reflectance spectra of the solvent-extracted meals, and to Mildred Murray for the spectrophotometric data on the solvent-extracted oils.

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Effect of Ultrasonic Waves On Oils

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ULTRASONIC waves have already found some important applications in science besides their well known development in the field of detection. One of their interesting effects is the possibility of heating bone marrow without affecting the bone. That suggested to the writer the idea of trying ultrasonic waves for the possible control of the silk worm pebrine, a parasite found in the eggs of the silk worm. With the help of K. Alexopoulos and D. Mannessis of the Physics department of the University of Athens, Greece, a small source of ultrasonic waves was constructed in 1938 in Athens, using electric potentials of 21,000 to 36,000 volts.

Unfortunately, the occupation of Greece by the German and Italian troops made it impossible for the writer to complete the experiment on the silk worm eggs and the only fact that he has been able to report is the hastening of germination of silk worm eggs by the effect of ultrasonic waves. It has been possible for him however, in the meantime, to carry on some experiments on oils and to study the effect of ultrasonic waves upon their acid value. The data obtained from these experiments are shown in Table 1. The oils used were refined seed oils and raw olive oils from different localities in Greece. The effect of ultrasonic waves, as seen from the results of these experiments, varies with the condition of the oil treated. With refined seed oils, as well as with pure oleic acid, a decrease in acid value was always observed. But with raw olive oils, the results were different. In some cases, a decrease was found, but more often an increase occurred and, in one case, no change at all. It is known that ultrasonic radiation activates the oxygen dissolved in water with the formation of H_2O_2 (1) and O_3 (2). It is possible therefore that with refined oils, where no intermediary oxidation products of the fatty esters occur except for small amounts of free fatty acids, the oxidation which takes place there affects the acids present and results in a decrease of the acid value of the oil. According to the various theories developed for the mechanism of the oxidation of oils by the oxygen, the first step in the process is the formation of an oxide or peroxide. This primary product of oxidation either breaks down directly into aldehydes and acids of lower molecular weight or reacts with water to form substances containing hydroxyl and Keto groups (3).

When, in addition to fatty acids, other more oxidizable substances like aldehydes and ketones are

TABLE 1.

Effect of Ultrasonic Waves Upon Various Samples of Refined and Raw Oils. (Acid Value mg. KOH/1 g. Oil.)

Samples	Before treatment	After 30 min. treatment	After 15 min. treatment	Remarks
A. Refined Oils				
1. Cotton Seed Oil	0.498	0.355	0.431	
2. Cotton Seed Oil	0.649	0.593	.....	
3. Soya Oil	0.244	0.184	0.187	
4. Sesame Oil	0.573	.....	0.557	No significant changes in fluorescence or iodine number were observed.
B. Raw Olive Oils				
1. Oil from the region of Crete	4.310	4.265	.....	
2. Oil from the region of Kymi	4.782	4.491	4.592	
3. Oil from the region of Argos	9.744	9.744	.....	
4. Oil from the region of Laconia	2.402	2.470	.....	
5. Oil from the region of Laconia	2.643	2.962	.....	
6. Oil from the region of Laconia	2.452	2.632	.....	
7. Oil from the region of Corfu	3.589	4.228	.....	
8. Oil from the region of Corfu	2.111	2.038	.....	
C. Oleic Acid	205.408	195.507	.....	