Processing of Cottonseed

1. Pigment Distribution in Oils and Meals Produced by Hydraulic and Screw Press Methods[1]

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Introduction

COTTONSEED is processed for oil in the United States by the hydraulic and continuous screw press methods. In both processes the seed is usually cooked for periods ranging from 30 minutes to one hour after which it is subjected to high pressures to express the oil. The amount of moisture added to the seed before or during cooking and the duration of cooking may vary greatly in different mills because they are based upon the conditions which have been found in practice to produce the maximum amount of oil from the type and quality of seed usually received at a given mill. In addition to these variations, processing of cottonseed by the hydraulic and continuous screw press methods may differ with respect to the amount of hulls left with the meats and the temperatures and pressures attained during expression of the oil. Hulls are frequently not removed prior to processing by the screw press method, and the temperatures and pressures during expression of the oil are usually higher than in the hydraulic press method.

In one of the earliest scientific investigations of the effect of processing on the pigmentation of cottonseed (1) it was observed that the amount of gossypol extractable with diethyl ether decreased markedly upon prolonged cooking of the kernels. Correlation of this observation with the absence of toxicity in processed cottonseed meal led to the theory that the gossypol in raw cottonseed reacts with the free amino groups of the protein of the surrounding tissue under the influence of heat to form "bound" gossypol. Subsequently, support for the theory of "bound" gossypol was found in the observation of Clark (2) that the compound, which forms when cooked cottonseed is extracted with hot aniline, is identical with dianilino-gossypol formed by the reaction of pure gossypol with aniline. In more recent investigations, Gallup (3) showed that the "bound" gossypol of cooked cottonseed does not account for all of the gossypol of the original uncooked seed. This investigator (4) also showed that cooked cottonseed of the same "free" or "bound" gossypol content could be produced by different conditions of cooking, and that these cooked seed did not necessarily produce the same physiological effects when fed to experimental animals. Gallup concluded from these observations that, during cooking of cottonseed, gossypol undergoes more complex changes than can be accounted for by its combination with the free amino groups of the protein of the seed.

Investigations of the pigmentation of cottonseed oils have likewise been limited to determinations of their gossypol content. Oils obtained by continuous screw pressing are reported to contain more gossypol than hydraulic-pressed oils (1, 5, 6), and the more compact foots and lower refining losses of the former oils have been attributed (6) to their higher gossypol content.

Nevertheless, it is quite apparent that the dark colors of crude cottonseed oils cannot be due entirely to the presence of the pale yellow gossypol. Moreover, the colors of the glands, in which the pigments of the raw seed are largely localized, range from a pale yellow to a deep purple color, and the more deeply colored glands are found to predominate in most samples of cottonseed. These observations may be presumed to indicate the presence of pigments other than gossypol. Recent investigations of cottonseed pigments have resulted in the isolation of three pigments in addition to gossypol. These are gossyfulvin (7), an orange-colored pigment; gossypurpurin (8), a purple cottonseed pigment; and gossycaerulin (9), a blue pigment which has been found only in cooked cottonseed. Solutions of these pigments exhibit characteristic and specific absorption in the visible and ultraviolet wavelength regions. Characteristic absorption bands occur at different wavelengths so that it is possible to measure the relative concentration of each pigment in any given solution or extract of the pigments in terms of the absorption at the wavelength of maximum absorption of each pigment. The instability of gossypol and the fact that the more recently isolated cottonseed pigments are derivatives of gossypol seemed to indicate that the latter might be converted to other pigments during the processing of cottonseed and that these conversion products might be responsible for the pigmentation of cottonseed products.

Recent investigations of the structure of cottonseed pigment glands demonstrated the existence of a wall enclosing the pigments. It was found that the gland wall is highly resistant to a variety of physical and chemical forces but is readily ruptured by the action of water (10, 11). Since the reactivity of the gland wall determines the behavior of pigments in stored seed (11), it seemed probable that it would also affect their behavior during cooking of the seed and expression of the oil.

The present investigation was undertaken with the objective of determining the nature of the changes which occur in gossypol and other pigments during the cooking and subsequent pressing of cottonseed by the hydraulic and continuous screw press methods. Because of the instability of the cottonseed pigments and the difficulty of reproducing actual processing conditions on a small scale the investigation was

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divided into two parts: (1) laboratory-scale experiments on the effect of cooking; (2) mill-scale experiments on the effect of cooking and pressing. The laboratory-scale cooking experiments were designed to simulate two extremes of moisture obtaining during mill-scale cooking of seed. Mill-scale experiments were run at two mills, at one mill by both the hydraulic and screw press methods and at the other by the hydraulic method only. Although the seed processed at the two mills were of similar origin and variety and the processing conditions by the two hydraulic press methods were approximately the same, the crude oils produced at the more southern mill were very much darker than those produced at the more northern mill. At the mill where seed was processed by both the hydraulic and continuous screw press methods the screw-pressed oils were more deeply colored than the hydraulic-pressed oils.

Methods

Absorption spectra of pure pigments. The absorption spectra of chloroform solutions of the known gossypol pigments are shown in Figure 1. Gossypol exhibits two principal absorption bands (12) in the wavelength region studied, one in the ultraviolet with maximum at 288 to 289 mμ, $E^{1\%}_{1\,cm.}$ 674; and the other in the near ultraviolet with maximum at 363.5 mμ, $E^{1\%}_{1\,cm.}$ 386. Gossyfulvin has two principal absorption bands (12), one in the ultraviolet, with maximum at 312 to 313 mμ, $E^{1\%}_{1\,cm.}$ 370.9; and the other in the visible with maximum at 439 to 440 mμ, $E^{1\%}_{1\,cm.}$ 649.4. Gossycaerulin has a single broad absorption band in the visible wavelength region (9) with maximum at 605 mμ, $E^{1\%}_{1\,cm.}$ 315.4.

Since the absorption spectrum of gossypurpurin had previously been determined (8) only in extracts of cottonseed, this pigment was prepared and its absorption spectrum determined after adequate purification. An ethereal extract of cottonseed was reextracted with N/2 ammonium hydroxide containing 10% sodium dithionite ($Na_2S_2O_4$), and the separated alkaline extract allowed to stand for one hour, whereupon an amber-colored solid separated. The alkaline suspension was treated with enough concentrated hydrochloric acid to reduce the pH to 8.4, and was then extracted with diethyl ether. Fifty ml. of glacial acetic acid was added to the ether extract, the mixture was heated on a steam bath for 30 minutes and allowed to stand overnight. The purple precipitate which formed was separated and washed by decantation with petroleum naphtha (Skellysolve F), transferred to a Buchner funnel and dried. In order to remove acetic acid a slurry of the compound in water was heated on a steam bath for two hours. The mixture was then freed of gossypol by repeated extraction with 95% ethanol, in which gossypol is very soluble and gossypurpurin only slightly soluble. Chloroform solutions of this purified preparation of gossypurpurin exhibited two absorption bands in the ultraviolet wavelength region, with maxima at 326 to 327 mμ, $E^{1\%}_{1\,cm.}$ 184.6, and 370 to 371 mμ, $E^{1\%}_{1\,cm.}$ 195.7; and two absorption bands in the visible wavelength region with absorption maxima at 530 mμ, $E^{1\%}_{1\,cm.}$ 186.7, and at 565 to 566 mμ, $E^{1\%}_{1\,cm.}$ 225.7.

Preparation of samples. In order to accomplish contact of all the pigment glands of the seed with the solvent all seed samples were very thoroughly comminuted. Large samples of seed were de-hulled

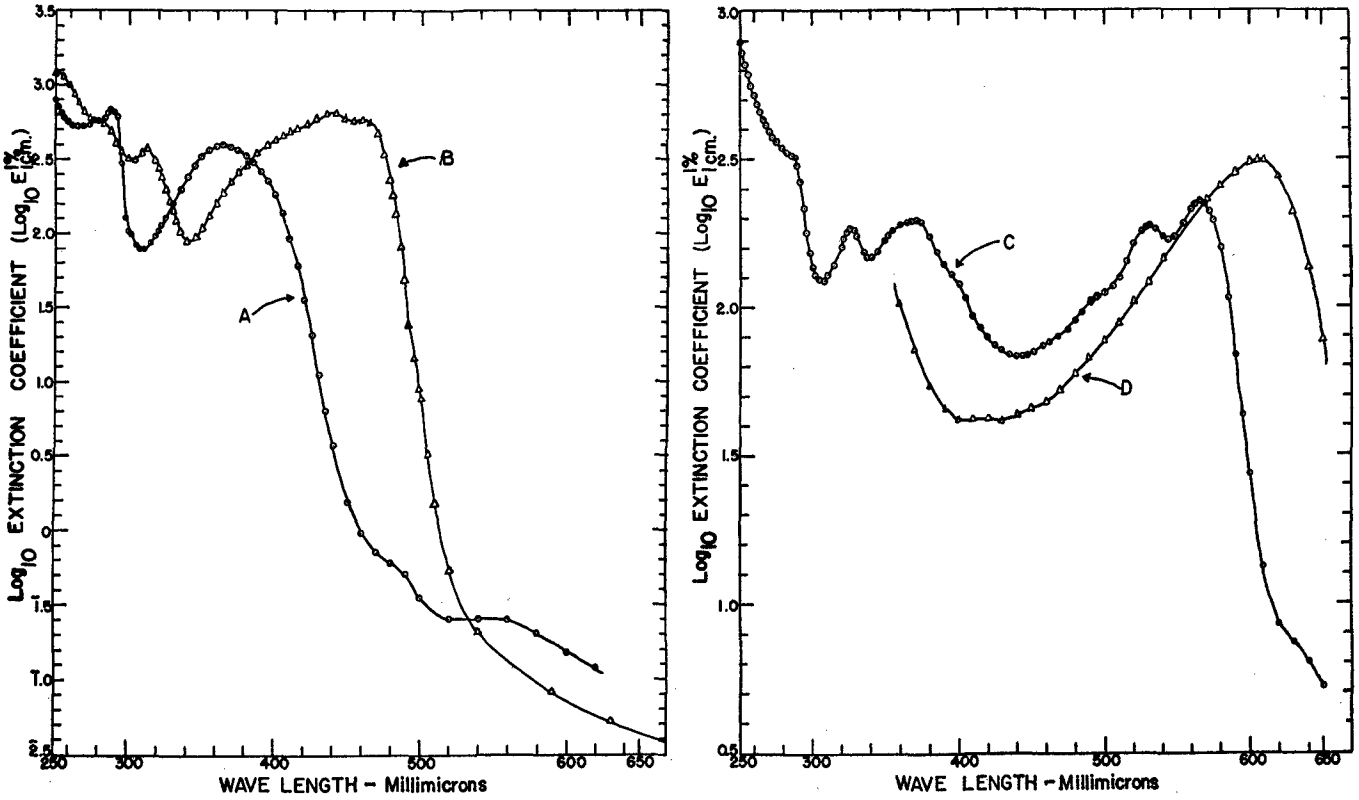

FIG. 1. Absorption spectra of pure cottonseed pigments.

A. Gossypol
B. Gossyfulvin
C. Gossypurpurin
D. Gossycaerulin

in a Bauer mill and then rolled to a thickness of 0.004 to 0.008 in. in flaking rolls. Smaller samples of seed were hulled in a Bauer mill, ground in a Burr mill, and then passed through a U. S. No. 50 sieve.

The meal samples were ground in a Burr mill and passed through a U. S. No. 50 sieve. The hulls were freed manually of as much of the adhering linters and kernel particles as possible and were then ground in a Wiley mill using a sieve having openings one millimeter in diameter. For determining the pigmentation of steamed hulls samples of the ground hulls were suspended in a current of steam for one hour.

The crude oils were refined according to official A.O.C.S. methods (13).

Determination of the pigmentation of seed, meal, and hull samples. It was shown in a previous investigation (14) by comparison of the gossypol content of chloroform extracts of cottonseed and cottonseed meal prepared by equilibration for 24, 48, and 72 hours at 38° F. that in each case the extracts contained identical amounts of gossypol. In the present investigation complete absorption spectra in the visible wavelength region of extracts prepared under the same conditions but in low-actinic glassware were found to remain constant for periods of extraction ranging from 24 to 72 hours. It was concluded that equilibration with chloroform for 24 hours at 38° F. extracts all of the pigments soluble in chloroform and that these pigments are stable in chloroform at 38° F. when protected from light, for a total of 72 hours. Therefore, for the determination of the pigment content of seed, meal, and hull samples weighed amounts of the finely ground products were equilibrated with measured volumes of chloroform for 24 hours at 38° F. in low-actinic glassware; the extracts were filtered and stored in low-actinic glassware at 38° F.; and their absorption spectra and gossypol contents determined within 48 hours after filtering.

Absorption spectra of the chloroform extracts were determined either with a Coleman double monochromator spectrophotometer or with a Beckman quartz spectrophotometer. The specific extinction coefficients $E^{1\%}_{1\,cm.}$ were calculated on the basis of one gram of product equilibrated with 100 ml. of chloroform, and a light path of 1 cm.

The content of gossypol of the seed extracts was determined by means of the antimony trichloride-spectrophotometric method (14). The relative concentrations of gossypurpurin and gossycaerulin were estimated on the basis of the specific extinction coefficients of the extracts at 570 and 610 mμ, respectively, using a Coleman monochromator spectrophotometer.

Determination of pigment content of oils. The absorption spectra of chloroform solutions of the crude, refined and bleached oils were determined with a Beckman quartz spectrophotometer, and their specific extinction coefficients, $E^{1\%}_{1\,cm.}$ were calculated.

Direct application of the antimony trichloride spectrophotometric method to the determination of the gossypol content of the crude oils was not possible because of the presence of excessive amounts of interfering substances. Therefore, an alkaline extraction method was developed for the quantitative isolation of gossypol from the non-acidic components of the oils. Chloroform solutions of pure gossypol were used for establishing optimum conditions for quantitative extraction and recovery of gossypol. Aqueous sodium hydroxide was found to be better than potassium or ammonium hydroxide, and the optimum concentration was found to be 0.05 to 0.1 molar. As the result of a series of experiments with a number of reducing agents in different concentrations, sodium dithionite ($Na_2S_2O_4$) in a concentration of 1% by weight in the sodium hydroxide solution was found to be adequate for preventing oxidative decomposition of gossypol in alkaline solution. Quantitative recovery of gossypol from the alkaline sodium gossypolate solutions was accomplished by mixing aliquots of alkaline extracts with measured volumes of chloroform and acidifying the mixtures with concentrated hydrochloric acid.

The alkaline extraction method was applied to the extraction of gossypol from the crude cottonseed oils; and the antimony trichloride spectrophotometric method applied to the chloroform solutions obtained from these extracts upon acidification. The specificity of the antimony trichloride reaction for gossypol was determined on the basis of ratios of the extinction coefficients at the points of characteristic absorption of the gossypol-antimony trichloride reaction product.[3] The sensitivity of the method could be increased appreciably by transferring the sodium gossypolate from relatively large volumes of alkali to relatively small volumes of chloroform.

The recently published aniline-spectrophotometric method for the determination of gossypol in crude cottonseed oils (15) was also applied to the crude oils.

Procedure

Laboratory-scale cooking of cottonseed. The effect of heat on the pigmentation of cottonseed was determined in a series of parallel experiments consisting of (a) whole seed, unmoistened, and (b) whole seed, moistened. The seed samples were heated in an autoclave at 10 to 12 lbs. pressure (116° C.) for periods of time ranging from five minutes to two hours. No additional moisture was added to one series of seed before heating. In the other series water was added in the amount of 100 ml. of water per 200 g. of seed. In order to obtain uniform distribution of the added moisture the seed was maintained in contact with the added water for a period of 18 hours before heating. The initial moisture content of the whole seed was 7.61% and that of the kernels of the whole seed after addition of moisture was 41%.

Parallel cooking experiments were carried out with two additional series of seed, one hulled and the other hulled and finely ground. In both series water in amount equal to that used in the first experiment was added to one set of samples prior to cooking and the other set was heated without addition of moisture.

In order to determine the effect on the pigment glands of moistening and heating free hand sections of the original and cooked seed were examined with the aid of a microscope. The color and condition of the glands and the surrounding tissue were noted.

Processing of seed by hydraulic press method. Mill-scale tests, designated 166 and 167, respectively, were carried out at two mills.

Processing of seed at mill 166 was carried out according to the following procedure. The seed, which contained 7.30% of moisture, was delintered and then decorticated. Sufficient hulls were then added to

[3] The absorption spectrum of the reaction product of antimony trichloride with pure gossypol has two well-defined absorption maxima at 380 and 520 mμ, and a minimum at 430 mμ so that it can be mathematically characterized. R_a, for pure gossypol, the ratio of the extinction at 520 mμ to that at 430 mμ, is 2.68 ± 0.23; R_b, the ratio of the extinction at 520 mμ to that at 380 mμ, is 1.22 ± 0.07 (14).

produce a press cake containing 42% of protein. Water was added to the mixture of hulls and kernels at the rate of 17 pounds of water per ton of seed. The moistened seed was then flaked and the flakes conveyed by means of a screw conveyor to the cooker. In the steam-jacketed cooker the flaked meats were cooked without further addition of moisture for a period of 50 minutes until they reached a temperature of 232° F. The meal was then formed into cakes, the cakes placed in the presses, and the oil expressed in the conventional method of operation.

Processing of seed at mill 167 was carried out according to a slightly different procedure. Seed having a moisture content of 8.87% was delintered, decorticated, and then flaked. The flaked seed was conveyed to a steam-jacketed cooker and moisture, in the form of live steam, was added to the seed in the top stack of the cooker. Cooking was continued for a total of 80 minutes by which time the temperature of the meal had been raised to 232° F.

The moisture contents and temperatures of the products at various stages of processing are assembled in Tables 1 and 2.

TABLE 1

Percentage of Moisture in Cottonseed Meats at Various Stages of Processing by Hydraulic and Screw Press Methods

Product	Hydraulic press method		Screw press method
	No. 166	No. 167	No. 166
Delinted seed, before milling	8.91	9.38	8.91
Flakes from rolls	12.55	.....	.....
Flakes or meal to cooker	11.30	8.87	7.13 [b]
Flakes, first stack of cooker [a]	.....	10.86	.....
Meal to former or press	7.46 [c]	7.54 [c]	3.92 [d]

[a] Maximum moisture content after addition of steam.
[b] Ground, undecorticated seed.
[c] To former.
[d] Entering expeller.

TABLE 2

Temperature of Cottonseed Products at Various Stages of Processing by Hydraulic and Screw Press Methods

Product	Hydraulic method	Screw press method
	°F.	°F.
Meal to press	232	224
Oil from press	140	180
Oil in press-trough	96-114	120

Processing of seed by screw press method. A mill-scale test on the screw press method was carried out at mill 166 at the same time as that on the hydraulic press method. In order to compare their pigmentation small samples of screw-pressed oil and meal were obtained from a nearby oil mill, designated Br.

Processing of cottonseed at mill 166 by the screw press method was carried out as follows: Part of the seed coming from the delintering machines was processed by the hydraulic press method as previously described, and part was ground without preliminary decortication and conveyed to a steam-jacketed cooker where it was cooked without addition of moisture for a period of one hour. The oil was expressed in Anderson No. I expellers. Temperatures and water content of seed and meal at various stages of processing are included in Tables 1 and 2.

The seed at the second mill, Br, was processed by the screw press method, as follows: The delintered seed was ground without decortication and conveyed to a steam-jacketed cooker where it was cooked for 45 minutes without prior addition of moisture. The temperature in the cooker ranged from 140° to 150° F. in the top stack of the cooker to 230° to 232° F. in the fourth or bottom stack. The only water added during cooking was that absorbed by the meats from live steam applied in the second stack of the cooker. In all other respects the processing conditions were the same as those at mill 166.

Sampling of seed, meal, and oil. Samples of the seed processed at mills 166 and 167 were taken at one-minute intervals over a period of one hour at the point where they emerged from the seed cleaner. The samples were designated CS 166 and CS 167, respectively. Their composition is shown in Table 3.

TABLE 3

Composition of Seed and Meal Processed by Hydraulic and Screw Press Methods

	Seed		Meal			
	166	167	166-PC	167-PC	166-EC	166-2-EC
Moisture, %	8.91[a]	9.38[a]	9.65	9.31	9.47	4.45
Lipids (ground seed), %	33.46[b]	31.98[c]	4.50	4.60	4.75	6.08
Free fatty acids in oil, %	0.5	0.6	0.9	1.4	1.8	.....
Nitrogen, %	3.33	3.42	6.03	6.48	6.68	4.47
Gossypol in kernels, %	1.13	0.92	.....	.....	.....	.....

[a] Moisture of undecorticated seed.
[b] Lipids undecorticated seed, 18.45%.
[c] Lipids undecorticated seed, 17.23%.

Sampling of the oil and meal at the two mills was carried out at such time intervals that the oil and meal corresponded to seed which had been sampled at the cleaner. Small samples of oil were collected from each press used in the experimental run. The oil was filtered and allowed to cool slowly overnight in order to approximate the rate of cooling in normal mill operation. The meal from the same battery of presses was sampled by breaking out one-eighth of one cake from each press. The collected samples were ground and the resultant meal thoroughly mixed before resampling. The oil samples from the two experiments were designated CS 166-HO and CS 167-HO, and the meal samples as CS 166 PC and CS 167 PC, respectively.

Samples of the oil and meal corresponding to the original seed samples were taken from the expeller press. These samples were designated CS 166 EO and CS 166 EC, respectively. No samples of seed were collected at mill Br corresponding to the oil and meal samples. The oil sample from this mill was designated Br-EO, and the meal Br-EC. The composition of the meal samples is shown in Table 3.

Results

Pigmentation of cooked seed. The relative concentrations of gossypol, gossypurpurin, and gossycaerulin in seed cooked without prior removal of hulls are shown in Table 4 and Figure 2. The amount

TABLE 4

Changes in Extractable Pigments During Cooking of Unmoistened and Moistened Cottonseed at 116° C.

Time of cooking, minutes	Unmoistened			Moistened [a]		
	Gossypol, %	Gossypurpurin, rel. conc.	Gossycaerulin, rel. conc.	Gossypol, %	Gossypurpurin, rel. conc.	Gossycaerulin, rel. conc.
0	1.18	0.0820	0.0188	0.804	0.0649	0.0124
5	0.835	0.0901	0.0346	0.293	0.0390	0.0151
10	0.795	0.106	0.0267	0.263	0.0387	0.0151
15	0.844	0.118	0.0333	0.298	0.0376	0.0207
20	0.715	0.107	0.0333	0.197	0.0404	0.0201
30	0.552	0.122	0.0422	0.220	0.0372	0.0244
60	0.376	0.154	0.0808	0.146	0.0253	0.0159
90	0.294	0.140	0.0878	0.119	0.0263	0.0197
120	0.260	0.147	0.0909	0.132	0.0256	0.0221

[a] Water added before cooking in the proportion of 100 ml. to 200 g. of seed.

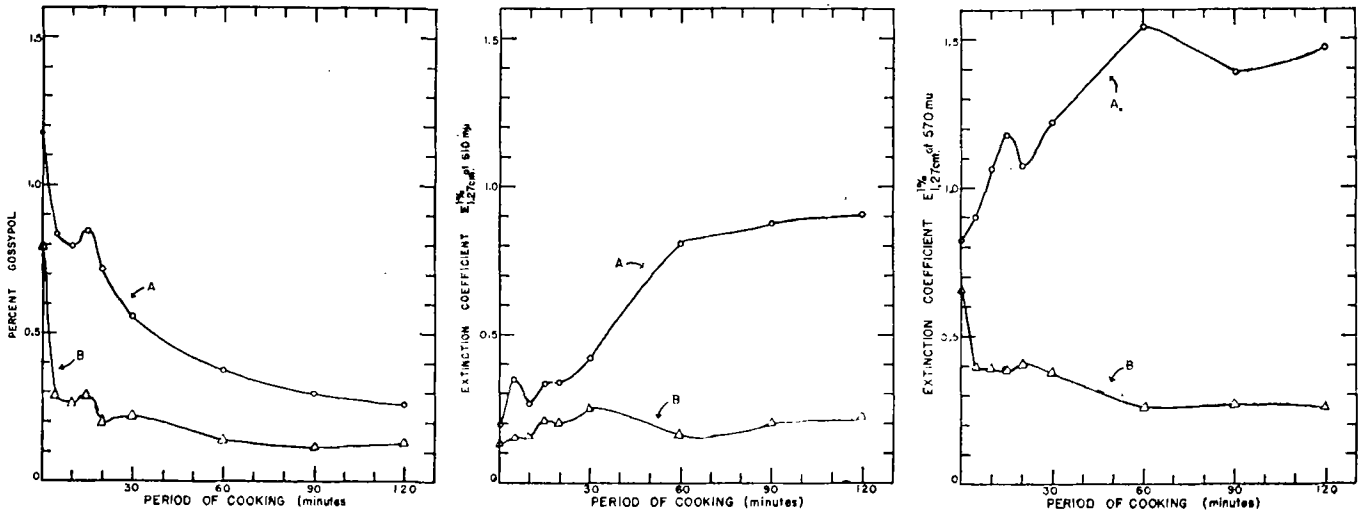

FIG. 2. Variation in extractable pigments during Heating of Cottonseed.

1. Gossypol — A. No moisture added before heating. B. Moisture added before heating.
2. Gossycaerulin — A. No moisture added before heating. B. Moisture added before heating.
3. Gossypurpurin — A. No moisture added before heating. B. Moisture added before heating.

of extractable gossypol decreased greatly during the initial stages of heating, and to a greater degree in the moistened than in the dry seed. Upon prolonged heating, however, the content of gossypol in the dry-cooked seed approached that in the wet-cooked seed. In the case of the unmoistened seed the content of gossypurpurin and gossycaerulin increased during heating as the gossypol content decreased. Changes in the content of gossypurpurin and gossycaerulin in moistened seed roughly paralleled the changes in the content of gossypol as the period of heating was increased.

The variations in gossypol content during cooking of dry and moistened decorticated seed, both whole and ground, paralleled those observed with undecorticated seed. During the heating of dry seed both gossypurpurin and gossycaerulin increased less rapidly in the decorticated than in the undecorticated seed. The content of extractable gossypurpurin and gossycaerulin decreased at approximately the same rate during heating of moistened seed regardless of whether it was undecorticated, decorticated, or decorticated and ground.

Physical distribution of pigments in cooked seed. Microscopic examination of sections of the cooked seed (Table 5) revealed that some of the pigment glands of the moistened seed were ruptured even before heating and most of the remaining glands were ruptured as the result of cooking whereas those of the unmoistened seed remained largely intact except after prolonged exposure to heat. The pigments of the ruptured glands were dispersed in the surrounding tissue.

Pigmentation of the seed prior to processing. The content of gossypol in the seed processed at mills 166 and 167 differed considerably from each other. One sample of seed, CS 166, contained 1.13% gossypol, while the other, CS 167, contained only 0.92% gossypol. The gossypurpurin contents of the two seeds (Table 6 and Figure 3) also differed considerably

TABLE 5

Effect on Cottonseed Pigment Glands of Dry and Wet Cooking [a]

Sample No.	Time of cooking, minutes	Condition of glands	Condition of tissue
D_0	0	Intact	Normal
D_5	5	Intact	Slightly dark, yellow in spots
D_{10}	10	Few broken	Slightly dark, yellow in spots
D_{15}	15	Few broken	Yellow around glands
D_{20}	20	Some partially broken	Yellow around glands
D_{30}	30	Some broken but still colored	Yellow around glands
D_{60}	60	Some emptied, some broken	Yellow around glands, other tissue darker than D_{30}
D_{90}	90	Many broken, some emptied completely	Red around glands, other tissue darker than D_{60}
D_{120}	120	Most emptied or broken	Dark red around glands, other tissue very dark
W_0	0	Most intact, but cloudy	Light colored, very brittle
W_{10}	10	Half intact, others broken, but still colored	Yellow around glands, very brittle
W_{20}	20	Most broken and partially emptied	Red around glands, darker than W_{10}, very brittle
W_{60}	60	Some still intact, but cloudy	Red around glands, darker than W_{20}, very brittle
W_{120}	120	Most completely emptied, few intact and cloudy, others partially emptied	Red around glands, other tissue very dark and very brittle

[a] D_0-D_{120} no water added prior to cooking, W_0-W_{120} water added before cooking in the proportion of 100 ml. of water to 200 g. of seed.

from each other. Comparison of the absorption spectra of extracts of both samples of seed with those of pure gossypol and gossypurpurin showed that these seeds contained no detectable amounts of extractable pigments other than gossypol and gossypurpurin.

Pigmentation of the hydraulic- and screw-pressed meals. Complete absorption spectra data of chloro-

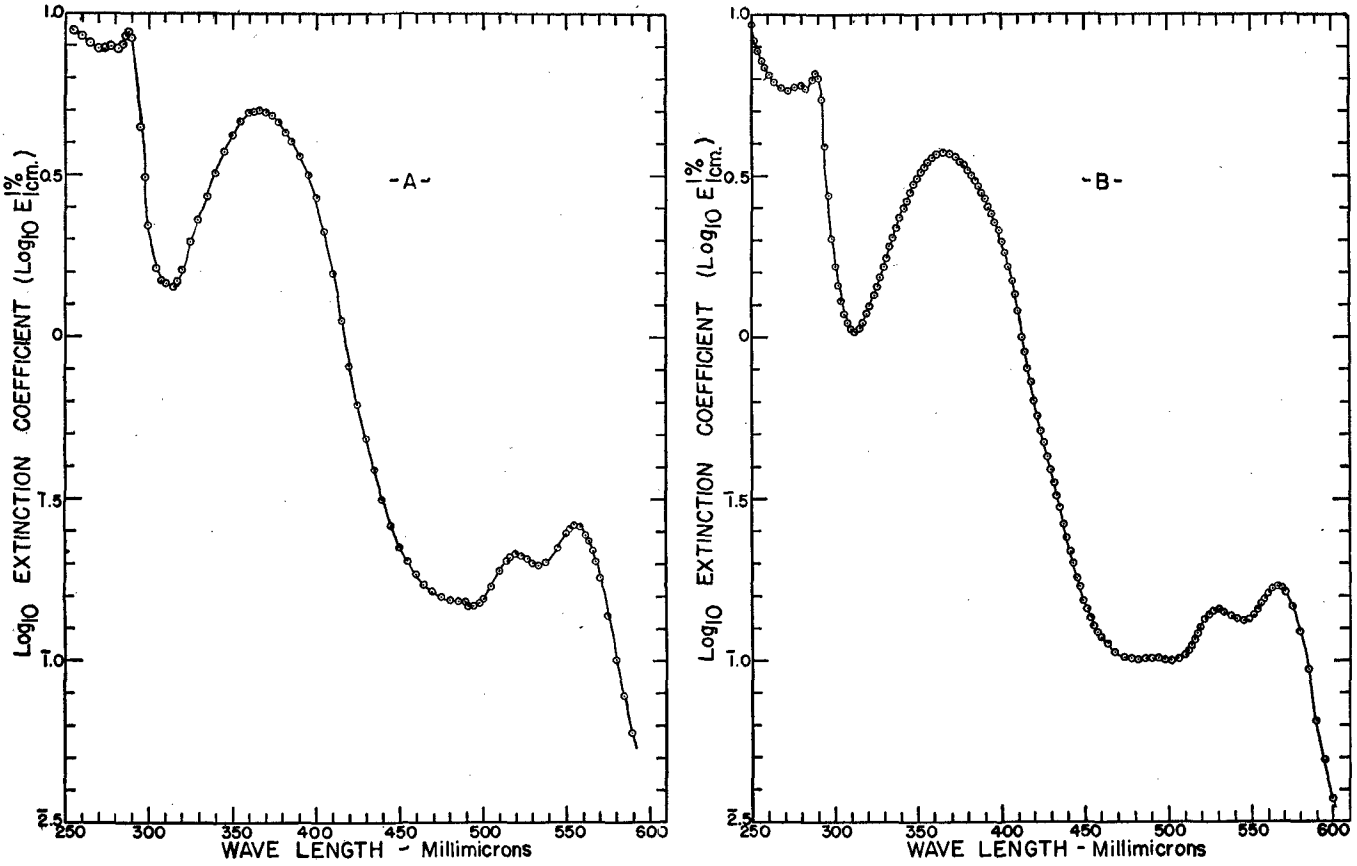

FIG. 3. Visible and ultraviolet absorption spectra of chloroform extracts of cottonseed.

A. CS-166 B. CS-167

TABLE 6

Extinction Coefficients of Chloroform Extracts of Cottonseed

Type of absorption	CS-166		CS-167	
	Wave-length	Ext. Coef.[a]	Wave-length	Ext. Coef.[a]
	mμ	$E^{1\%}_{cm.}$	mμ	$E^{1\%}_{cm.}$
Min	273	7.70	272	5.80
Max	279	7.97	278-9	6.00
Min	282	7.83	282	5.88
Max	288	8.78	288	6.56
Min	315	1.42	313	1.03
Max	364-9	5.00	364-6	3.73
Max	530-1	0.213	530-2	0.144
Min	544-6	0.198	545-6	0.133
Max	566	0.261	564-6	0.170

[a] Per cent expressed in terms of weight of ground seed treated with chloroform.

form extracts of the hydraulic- and screw-pressed meals (Table 7 and Figure 4) indicate the presence of gossypurpurin in these products and a pigment having an absorption maximum at 368 to 374 mμ, similar to that of gossypol. The inflection at 420 to 440 mμ (Figure 4) indicates the presence of a third pigment with an absorption band in the 420 to 440 mμ wavelength region. The absorption spectra of the antimony trichloride reaction products of chloroform extracts of the meals were not characteristic of pure gossypol. From this observation it can be assumed that the concentration of gossypol was so slight and that of other pigments so great that the gossypol-antimony trichloride reaction was completely masked by interfering substances.

Refining characteristics of oils. The hydraulic-pressed and screw-pressed oils were refined and bleached according to the official refining loss and bleach color methods of the American Oil Chemists' Society with the results shown in Table 8. The oils from the more highly pigmented seed, CS 166, were more deeply colored than those from the less highly pigmented seed CS 167. Both of the screw-pressed oils were much more highly colored than the hydraulic-pressed oils.

Pigmentation of crude oils. Complete absorption spectra of chloroform solutions of the crude hydrau-

TABLE 7

Extinction Coefficients of Chloroform Extracts of Hydraulic- and Screw-Pressed Meals

Type of absorption	166-PC		167-PC		166-EC		166-2-EC[a]		Br-EC	
	mμ	$E^{1\%\ b}_{cm.}$	mμ	$E^{1\%\ b}_{cm.}$	mμ	$E^{1\%}_{cm.}$	mμ	$E^{1\%}_{cm.}$	mμ	$E^{1\%}_{cm.}$
Max	372-5	0.240	370-6	0.111	368-74	0.200	372-80	0.138	372-8	0.190
Max	526-30	0.0217	524-32	0.0135	528-30	0.0347	526	0.0201	527-32	0.0240
Min	546-8	0.0201	546-7	0.0125	544-6	0.0323	548	0.0180	545-6	0.0221
Max	566	0.0228	562-4	0.0135	565-7	0.0388	565-6	0.0200	564-8	0.0257

[a] Also exhibited a minimum inflection at 510 mμ, at which $E^{1\%} = 0.0188$.

[b] Per cent expressed in terms of weight of meal treated with chloroform.

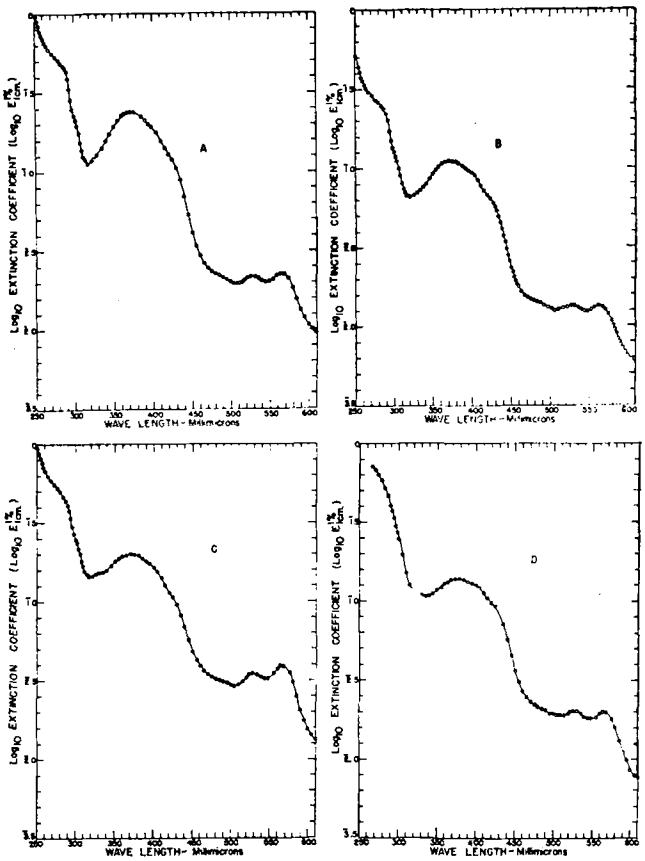

FIG. 4. Visible and near ultraviolet absorption spectra of chloroform extracts of cottonseed meals.

A. CS-166-PC B. CS-167-PC
C. CS-166-EC D. CS-166-2-EC

lic-pressed oils showed (Table 9 and Curves A and B of Figure 5) that these oils contained similar pigments having well defined absorption characteristics but differing markedly from gossypol. The existence of two absorption maxima at 380-382 mμ and 398-403 mμ indicated the probable presence of at least two principal pigments in the crude oils. The more deeply colored oil, CS 166-HO, obtained from the more deeply pigmented seed, contained more of the crude oil pigments than CS 167-HO.

TABLE 9

Extinction Coefficients of Crude Hydraulic- and Screw-Pressed Oils in Chloroform Solution

Crude oil No.	Position of maximum mμ	Extinction coefficient $E^{1\%}_{1cm.}$
CS-166-HO	381-2 401-3	0.574 0.561
CS-167-HO	380-2 398-400	0.158 0.154
Br-EO	367-8	3.00
CS-166-EO	368-71	2.22
CS-166-2-EO	370-2	1.42

Complete absorption spectra of chloroform solutions of the crude screw-pressed oils (Table 9 and Curves A and B of Figure 6) possessed a single maximum at 367-371 mμ indicating the probable presence of only one principal pigment in these oils, in contrast to the two pigments observed in crude hydraulic-pressed oils.

Since the absorption spectra of the antimony trichloride reaction products of the crude oils were not characteristic of gossypol, the crude oils were extracted with aqueous alkali in order to isolate gossypol from interfering non-acidic components.

The materials removed by dilute aqueous alkali from screw-pressed oils, transferred to chloroform by acidification and agitation, did not produce a typical gossypol-antimony trichloride reaction product. The ratios, R_a and R_b, were 1.22 and 0.788, respectively, and the product was unstable. The pigments remaining in the oil after extraction by alkali did not give a typical gossypol-antimony trichloride test. The ratios, R_a and R_b, were 0.656 and 0.436, respectively.

Alkaline extraction of hydraulic-pressed oil did not remove sufficient pigment to give a visible reaction

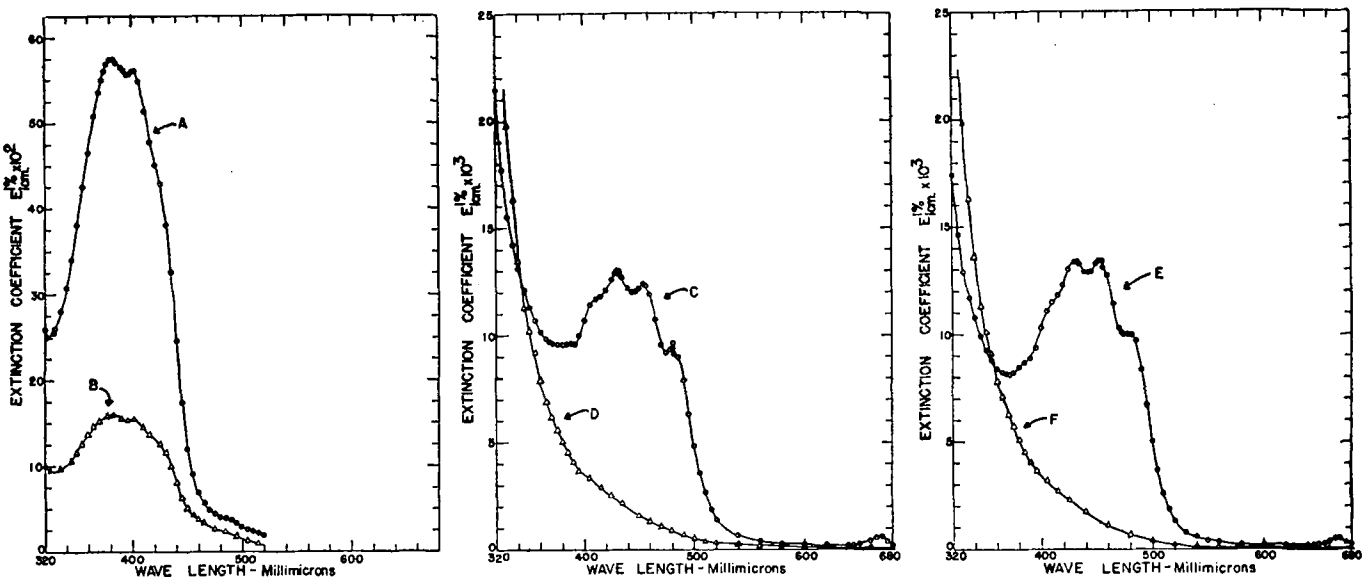

FIG. 5. Visible and near ultraviolet absorption spectra of hydraulic-pressed cottonseed oils.

A. CS-166-HO-Crude C. CS-166-HO-Refined E. CS-167-HO-Refined.
B. CS-167-HO-Crude D. CS-166-HO-Bleached F. CS-167-HO-Bleached.

TABLE 8

Refining Data on Oils

Sample No.	H_2O %	FFA %	Refining loss, per cent lye strength, Bé°				Lovibond color					
							Crude		Refined		Bleached	
			12°	14°	16°	20°	Y	R	Y	R	Y	R
166-HO	0.07	0.74	4.1	4.3	.....	.....	70	18.3	35	5.6	20	1.9
167-HO	0.07	0.89	6.1	6.4	.....	.....	70	10.8	35	5.2	20	1.2
166-EO	.....	1.0	.....	.....	7.6	.....	.....	.....[a]	35	10.3	35	4.2
166-2-HO	.....	1.2	6.6	6.4	.....	.....	.....	.....[a]	35	6.8	20	2.8
167-2-HO	.....	1.0	4.0	4.0	.....	.....	70	22.5	35	5.7	20	1.3
166-2-EO	.....	1.5	.....	.....	13.2	19.6	.....	.....[a]	35	20.3	35	10.2

[a] Too dark to read.

with antimony trichloride. These results indicate that none of the pigments present in hydraulic- and screw-pressed oils is gossypol.

On the other hand, application of the aniline- spectrophotometric method of Smith (15) indicates the presence of gossypol in both hydraulic- and screw-pressed oils (Table 10). Since no gossypol could be

TABLE 10

Gossypol in Crude Oils According to Aniline Spectrophotometric Method

Crude oil No.	Gossypol,[a] per cent	Extinction coefficient at 365 mμ $E^{1\%}_{cm.}$ [b]	
		Cal'd [c]	Det'd
CS-166-HO	0.0456	0.176	0.570
CS-167-HO	0.0303	0.117	0.145
CS-166-2-EO	0.280	1.08	1.39
Br-EO	0.610	2.35	2.98

[a] Determined by the Method of F. H. Smith, Ind. Eng. Chem., Anal. Ed., *18*, 41-43 (1946).

[b] No maximum exhibited at 365 mμ.

[c] Calculated on basis (a) as product of weight of gossypol in 1 g. of crude oil and $E^{1\%}_{1cm.}$ of pure gossypol in chloroform at 365 mμ.

isolated from these oils with aqueous alkali, it would seem that aniline reacts with crude oil pigments other than gossypol. Moreover, even if the aniline reaction were assumed to be specific for gossypol, the calculated values of the absorption due to gossypol in the crude oils, as shown in Table 10, would show very poor correlation with the actual absorption exhibited by the crude oils.

TABLE 11

Extinction Coefficients of Refined Hydraulic-Pressed Oils in Chloroform

Refined oil No.	Position of *maximum* mμ	Extinction coefficient $E^{1\%}_{1cm.}$
CS-166-HO	410-415 [a]	0.0117
CS-167-HO	405-415 [a]	0.0111-0.0118
CS-166-HO	430-431	0.0130
CS-167-HO	430-434	0.0134
CS-166-HO	455	0.0124
CS-167-HO	452-455	0.0134
CS-166-HO	480-481	0.00926
CS-167-HO	474-482 [b]	0.0100

[a] Position of inflection.

[b] Position of shoulder.

Pigmentation of alkali-refined oils. The absorption spectra of the alkali-refined, hydraulic-pressed oils (Table 11 and Curves C and E of Figure 5) revealed the presence of pigments having characteristic and specific absorption. Oil, CS 166-HO, showed absorption maxima at 430-431 mμ, 455 mμ, and 480-481 mμ; oil CS 167-HO, exhibited absorption maxima only at 430-434 mμ and at 452-455 mμ. Although the spectral curves of the alkali-refined oils are of such well defined shape as to suggest the presence of a single pigment of carotenoid-like nature, the differences in the absorption spectra of the two oils precludes the possibility of the presence of only a single pigment in the alkali-refined oil. It seems probable, however, that only a very limited number of pigments are responsible for the color of alkali-refined, hydraulic-pressed oils.

In contrast to the refined hydraulic-pressed oils the screw-pressed oils were more highly colored and their absorption spectra showed no clearly defined absorption maxima (Curve C, Figure 6) but only a series of inflections, indicating the presence of a large number of pigments, probably the result of decomposition of the pigment originally present in the crude oil.

Pigmentation of bleached hydraulic- and screw-pressed oils. As shown in Curves D and F of Figure 5 no characteristic spectral absorption maxima were observed in the visible wavelength region of the bleached hydraulic-pressed oils. Comparison of the absorption spectra of the alkali-refined oils and those of their corresponding bleached oils indicates that the pigments in the alkali-refined oil having absorption maxima at 430-431 mμ, 455 mμ and 480-481 mμ are almost completely adsorbed and removed by the bleaching clay.

The absorption spectra of the bleached screw-pressed oils (Curve D, Figure 6) are similar to those of the bleached hydraulic-pressed oils, with no characteristic absorption maxima. However, the bleached screw-pressed oils are more deeply colored than the bleached hydraulic-pressed oils.

Pigmentation of hulls. Absorption spectra of chloroform extracts of cottonseed hulls showed that they contained very small amounts of gossypol and gossypurpurin. The content of extractable gossypol of steamed hulls was 0.000332%, that of unsteamed hulls was 0.000129%. The $E^{1\%}_{1cm.}$ at 565 mμ of chloroform extracts of the unsteamed hulls was 0.0042. Extracts of steamed hulls exhibited no absorption maximum at 565 mμ, probably because of the decomposition of gossypurpurin during the steaming of the hulls. As shown by Curves E and F in Figure 6, hot cottonseed oil extracts very little pigment from either steamed or unsteamed hulls. In view of the difficulty of removing all traces of kernels in the preparation of the hulls, it is quite probable that the small amounts of pigments observed in the extracts arose from contamination with kernel particles rather than from the hulls themselves.

Discussion of Results

The changes in gossypol which occur during the heating of unmoistened cottonseed are summarized in Figure 7. Under the influence of heat the gossypol within the intact glands is slowly converted principally to gossypurpurin and gossycaerulin. These

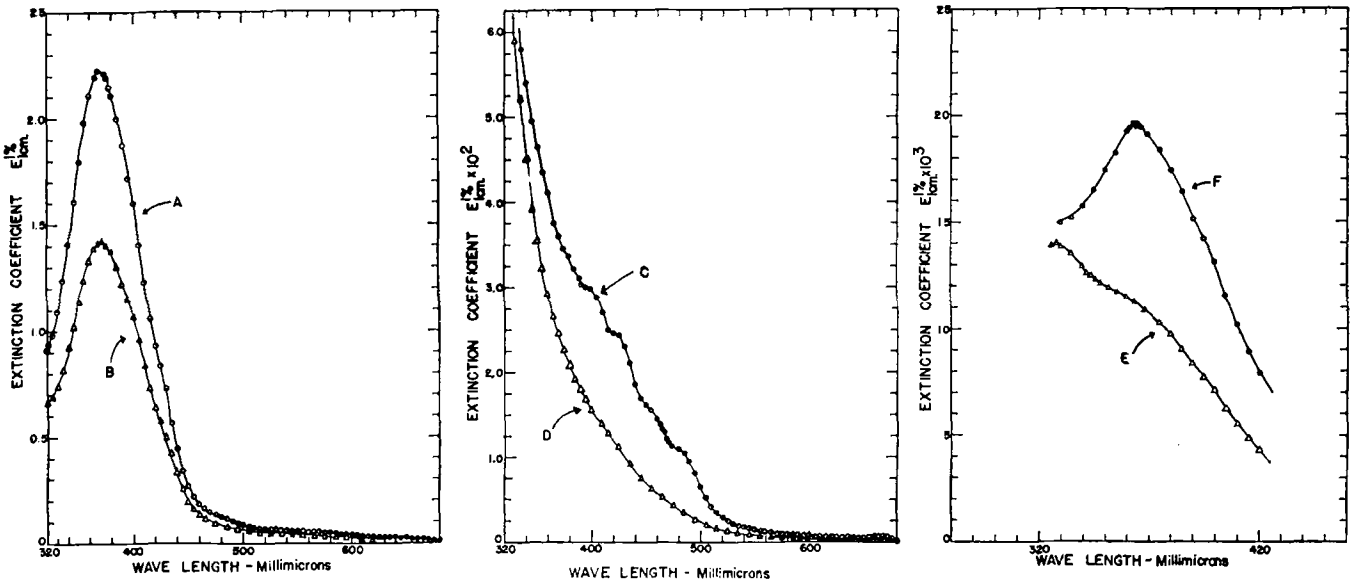

FIG. 6. Visible and near ultraviolet absorption spectra of screw(expeller)-pressed cottonseed oils and cottonseed hulls extracts.

A. CS-166-EO-Crude
B. CS-166-2-EO-Crude
C. CS-166-2-EO-Refined
D. CS-166-2-EO-Bleached
E. CS-166-Steamed hulls
F. CS-166-Hulls not steamed.

compounds are unstable and are in turn further decomposed by the action of heat. That gossypurpurin and gossycaerulin are not oxidation products of gossypol is suggested by the observation that they are formed in greater amounts during the heating of whole seed than during the heating of decorticated or finely ground seed.

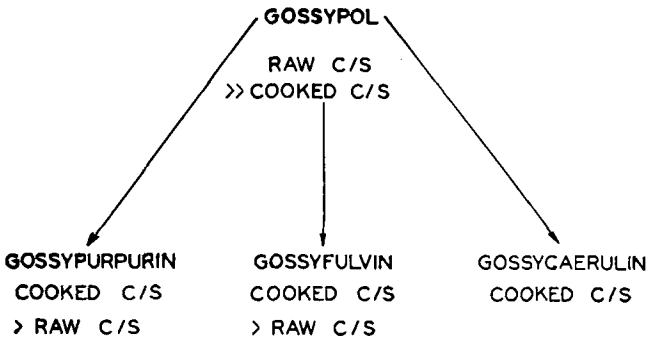

FIG. 7. Conversion of gossypol into other pigments during cooking of cottonseed.

When cottonseed is moistened prior to heating, some of the glands rupture immediately while others rupture only upon subsequent application of heat. During the initial period of heating all or most of the remaining pigment glands are ruptured and the pigments expelled into the surrounding tissue. Once the pigments have come into contact with the tissue, they apparently undergo very little further change or they are converted into compounds having low tinctorial properties, *i.e.*, they exhibit very little absorption in the visible wavelength region, or they are so strongly absorbed on the tissue that they can no longer be extracted with chloroform.

It is quite evident, however, that the changes which the very complex pigment, gossypol, can undergo in the very reactive surroundings of the cottonseed tissue are, themselves, very complex and cannot all be explained on the basis of our incomplete knowledge of the properties of cottonseed and its pigments. For example, the formation of gossycaerulin during the heating of cottonseed has been shown to be correlated with the pH of the seed. In view of its formation *in vitro* from gossypol under alkaline conditions, it is probable that the formation of gossypurpurin during the heating of cottonseed is correlated with the alkalinity of the seed. Finally, although gossyfulvin could not be detected in any of the seed or meal investigated here, it has been detected in appreciable amounts in some hydraulic-pressed meals (7, 14).

Similar changes in pigmentation evidently occur during the cooking of cottonseed on a commercial scale so that the absorption spectra of chloroform extracts of the commercial meals are very similar to those of the laboratory-scale cooked seed.

On the other hand, the pigments of the oils are very different from those of the meals, and the cooked seed.

Gossypol was found only in very small amounts in the screw-pressed oils, and not at all in the hydraulic-pressed oils. This was to be expected since it had been shown (16, 17) that gossypol is not stable in cottonseed oil at the elevated temperatures at which cottonseed is processed by either the hydraulic or screw press methods. However, it seems evident that the pigments of the oils must have been formed from one or more of the pigments in the cooked seed during expression of the oil. Since the seed of higher gossypol and gossypurpurin content yields crude oils of higher pigment content, one or both of these seed pigments is probably the precursor of the crude oil pigments.

Since the same seed was processed by both the screw and hydraulic methods, differences in pigmentation of the crude oils must be attributed to differences in processing conditions. For the screw press process hulls were not removed and no moisture was added prior to cooking, and both temperature and pressure during expression of the oil was higher in the expeller presses than in the hydraulic presses. Since it had previously been shown that the cottonseed hull pigments comprise only lignin and a xylan

body (18), it was to be expected, as had been shown, that the hulls do not contribute to the color of the oil. Moreover, it does not seem probable that the higher temperatures of the expeller press should be of great significance since they are neither as high nor as prolonged as during the cooking of cottonseed. The higher pressures during expression might account for the higher pigmentation of the screw-pressed oils but could hardly account for its complete difference from that of the hydraulic-pressed oils. Therefore, by the process of elimination and on the basis of the results of the laboratory-scale cooking experiments, it seems probable that the difference in the pigments of the crude expeller oils and the crude hydraulic-pressed oils must be attributed to the difference in the moisture contents of the seed during cooking. The pigments remain in the glands during the cooking of the expeller meal and so undergo a different series of changes than those of the ruptured glands in the hydraulic-pressed meal. When pressure is applied during expression of the oil, the glands are ruptured and some of the pigments are dissolved in the crude oil. It should be possible to obtain screw-pressed oils of the same pigment content as hydraulic-pressed oils by moistening the seed before or during cooking.

The acidic nature of gossypol coupled with the observation that alkali refining of crude oils removes not only the free fatty acids but much of the pigmentation, has led to the assumption that gossypol is the principal pigment of crude cottonseed oils. Although the crude oils contained no gossypol, the absorption spectra of alkali-refined oils showed that the pigments of the crude oils behave like gossypol with respect to their removal during alkali refining.

The observation that the crude hydraulic-pressed oil containing greater quantities of pigments with absorption maxima at 380-382 mμ and 398-403 mμ yields an alkali-refined oil containing less of the pigments which exhibit absorption maxima at 430-434 mμ, 452-455 mμ, and 480-482 mμ, whereas the less highly pigmented crude oil yields a more highly pigmented refined oil, suggests that the pigments of the alkali-refined oils are not formed during the refining process but are present in the original crude oils. It seems probable that their absorption in the crude oils is masked by that of the principal crude oil pigments, but when these pigments are removed by refining, the absorption spectra of the less concentrated or less deeply colored pigments which remain in the refined oil are readily measured. The differences in the relative heights of the absorption maxima in the different alkali-refined hydraulic-pressed oils indicate the presence of more than one pigment in these oils. However, the well-defined shape of the curves suggests the presence of a limited number of pigments.

On the other hand, the absorption spectra of the alkali-refined screw-pressed oils exhibit only a series of inflections and plateaus so that the curves appear to be composed of absorption similar to that of the alkali-refined hydraulic-pressed oils superimposed on absorption representing a large number of compounds. It would appear that the screw-pressed crude oils contain one principal pigment which undergoes oxidative decomposition upon contact with alkali, and, in addition, the pigments present in hydraulic-pressed oils which are not removed during alkali refining.

The lack of any characteristic absorption bands in the spectra of the bleached oils precluded the possibility of applying spectrophotometric analysis to the identification of the pigment or pigments responsible for the color of bleached oils.

Summary

1. The pigmentation of cooked cottonseed has been shown to depend principally upon the moisture content and period of heating of the seed.

2. Several samples of crude hydraulic-pressed and screw-pressed oils produced under known processing conditions were found to differ markedly from each other with respect to their original colors and refining characteristics.

3. The screw-pressed crude oils were more deeply colored and contained one principal pigment, whereas the hydraulic-pressed oils contained two principal pigments.

4. The absence of significant amounts of gossypol in the crude oils has been demonstrated by means of a new technic for the quantitative isolation of gossypol.

5. The crude oil pigments differed from gossypol, but like gossypol, they were removed during alkali refining.

6. The pigmentation of the crude oils has been shown to depend principally upon the pigmentation of the original seed and the moisture content of the seed during cooking.

7. On the basis of their absorption spectra it has been deduced that the alkali-refined hydraulic-pressed oils contain two to three pigments originally present in the crude oils whereas the alkali-refined screw-pressed oils contain these same pigments as well as a large number of decomposition products of the principal crude oil pigment.

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REFERENCES

1. Carruth, F. E., J. Biol. Chem., *32*, 87-90 (1917).
2. Clark, E. P., J. Biol. Chem., *76*, 229-235 (1928).
3. Gallup, W. D., Ind. Eng. Chem., *19*, 726-728 (1927).
4. Gallup, W. D., Ind. Eng. Chem., *20*, 59-63 (1928).
5. Royce, H. D., and Lindsay, F. A., Jr., Ind. Eng. Chem., *25*, 1047-1050 (1933).
6. Owen, G. W., Oil and Soap, *14*, 149-151 (1937).
7. Boatner, C. H., Caravella, M., and Samuels, C. S., J. Am. Chem. Soc., *66*, 838 (1944).
8. Boatner, C. H., Oil and Soap, *21*, 10-15 (1944).
9. Boatner, C. H., Samuels, C. S., Hall, C. M., and Curet, M. C., J. Am. Chem. Soc., (In press).
10. Boatner, C. H., and Hall, C. M., Oil and Soap, *23*, 123-128 (1946).
11. Boatner, C. H., Hall, C. M., Rollins, M. L., and Castillon, L. E., Bot. Gaz., (In press).
12. Boatner, C. H., O'Conner, R. T., Samuels, C. S., and Caravella, M., J. Am. Chem. Soc., (In press).
13. Official and Tentative Methods of the American Oil Chemists' Society. Revised to January 1, 1944.
14. Boatner, C. H., Caravella, M., and Kyame, L., Ind. Eng. Chem., Anal. Ed., *16*, 566-572 (1944).
15. Smith, F. H., Ind. Eng. Chem., Anal. Ed., *18*, 41-43 (1946).
16. Podolskaya, M., and Tobler, L., Masloboina Zhirovoe Delo, *16*, 5-7 (1940); C. A., *35*, 924 (1941).
17. Royce, H. D., Oil and Soap, *10*, 183-185 (1933).
18. Markley, K. S., J. Am. Soc. Ag., *20*, 1102-1107 (1928).