Influence of Condition of Seed on the Spectral Properties of Crude and Processed Hydraulic Cottonseed Oils[1]

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THE influence of processing on the spectral properties of a number of vegetable oils was described in a recent paper from this laboratory (2). The spectra were interpreted to indicate the pigments responsible for color in the oils and to indicate the fate of these pigments during processing of the oils. The cottonseed oil examined in the earlier work was a commercial hydraulic-pressed sample. The purpose of this paper is to report some investigations on the influence of "condition" and of storage of cottonseed on the spectral properties of the crude oils obtained by hot and cold hydraulic pressing and of refined, bleached, and deodorized oils obtained from them.

Oils

Seven crude cottonseed oils were obtained for the investigation. Some properties of these oils and of the refined, bleached, and deodorized oils processed from them are given in Table I. Numbers 1, 2, and 3 were commercial crude hydraulic oils containing 1.0, 6.4, and 9.7% free fatty acids, respectively. Seed representative of the lots from which oils Nos. 1 and 2 were pressed were supplied by the commercial mills. These seed were experimentally processed by use of pilot-plant equipment, under conditions simulating commercial practices, except that no heat was used. The oils were designated as Nos. 1a and 2a. Oils Nos. 4 and 5 were obtained from seed, prime when purchased, which had been stored 15 and 58 months, respectively, at low moisture contents without any attention given to temperature control. Both of these oils were extracted from the meats by hydraulic pressing, as in the case of oils 1a and 2a, without the application of heat, and were included to show the influence of extended storage of the seed before processing.

Each of the seven oils was refined, bleached, and deodorized by conventional laboratory methods. The Lovibond color values of the processed oils are included in Table I. Spectra of the 28 samples of oil were obtained from 220 mμ in the far ultraviolet through the visible region of the spectra to 720 mμ at the edge of the infrared with a Beckman Quartz Model DU spectrophotometer.[3] Isooctane was used as the solvent for the oils except that admixtures of ethanol and isooctane were used in three instances. The spectra of oil No. 1a were measured from 220 mμ to 410 mμ with 4% ethanol in isooctane, of oil No. 2a throughout in 10% ethanol in isooctane, and of oil No. 5 from 220 mμ to 420 mμ in 10% ethanol in isooctane. The spectral data are presented as curves of the logarithm of the extinction coefficients as the ordinate and the wavelength in millimicrons as the abscissa in Figures 1-5. The extinction coefficient as used in this report is defined as equal to the optical density (O.D.) as read from the instrument divided by the product of the concentration of the oil solution expressed as grams per liter (c) and the cell length expressed in centimeters (1): Extinction coefficient = O.D./cl.

The extinction coefficients ($\times 10^3$) at selected wavelengths, characteristic of the individual constituents of the crude oils, are given in Table II.

Results

Far Ultraviolet. The far ultraviolet absorption of cottonseed oil is characterized by bands with maxima at 232 and 268 mμ. These bands are due to diene and triene conjugation, respectively. It is generally agreed now that this so-called preformed conjugation is a result of oxidation of the oil (2, 5). In the spectra of cottonseed oil the magnitude of these bands is augmented somewhat by overlapping from the absorption of gossypol, which has absorption bands in these regions (4). This overlapping absorption must be kept in mind when interpreting the effects of refining and bleaching on the spectral properties.

The band at 232 mμ is due, in all probability, to small contributions from gossypol, whose concentration is low in these oils (Table II), and to oxidation-conjugation. The magnitude of this band in the spectra decreases during refining and bleaching, owing to the removal of gossypol and gossypol-like pigments. The cold-pressed oils (Figures 1a, 2a, 4 and 5) exhibit an increase in absorption during both refining and bleaching. In these cases the contribution to absorp-

TABLE I

Some Characteristics of Hydraulic Cottonseed Oils

No.	Seed processing	Type of processing	F.F.A. %	Gossypol[1] %	Refining			Lovibond color		
					Lye used		Loss %	Refined Y/R	Bleached Y/R	Deodorized Y/R
					%	Bé°				
1	Commercial	Hot	1.0	0.076	80	12	7.6	70/6.7	15/1.2	10/1.1
1a	Pilot plant	Cold	0.8	0.015	80	14	2.6	35/3.8	10/0.6	10/0.4
2	Commercial	Hot	6.4	0.097	Max.	22	19.5	70/10.3	70/3.8	20/2.3
2a	Pilot plant	Cold	2.8	0.053	80	12	6.9	70/6.6	35/1.2	10/0.6
3	Commercial	Hot	9.7	0.120	Max.	20	23.7	70/21.6	70/8.8	35/4.9
4	Pilot plant	Cold	1.2	0.007	80	20	2.7	35/1.5	10/0	3/0.5
5	Pilot plant	Cold	6.7	0.005	Max.	26	40.0	35/4.3	5/0.4 35/0.9 10/1.9	20/1.4

[1] By method of Pons, *et al.* (3).

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[3] Mention of names of firms or trade products does not imply that they are endorsed or recommend by the U. S. Department of Agriculture over other firms or similar products not mentioned.

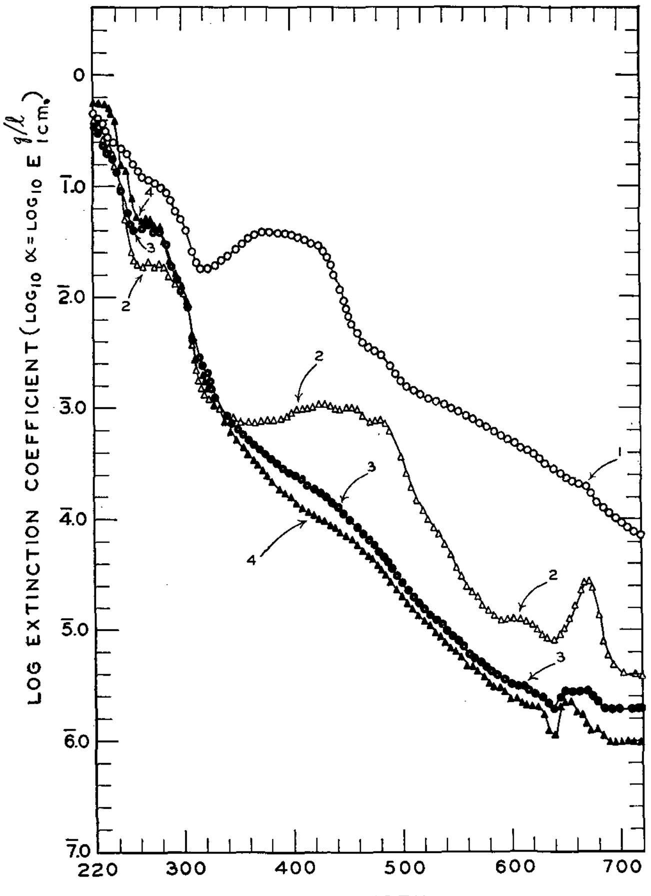

FIG. 1. Spectra of commercial hydraulic cottonseed oil containing 1.0% free fatty acid.

1. Crude.
2. Alkali-refined.
3. Alkali-refined and bleached.
4. Alkali-refined, bleached, and deodorized.

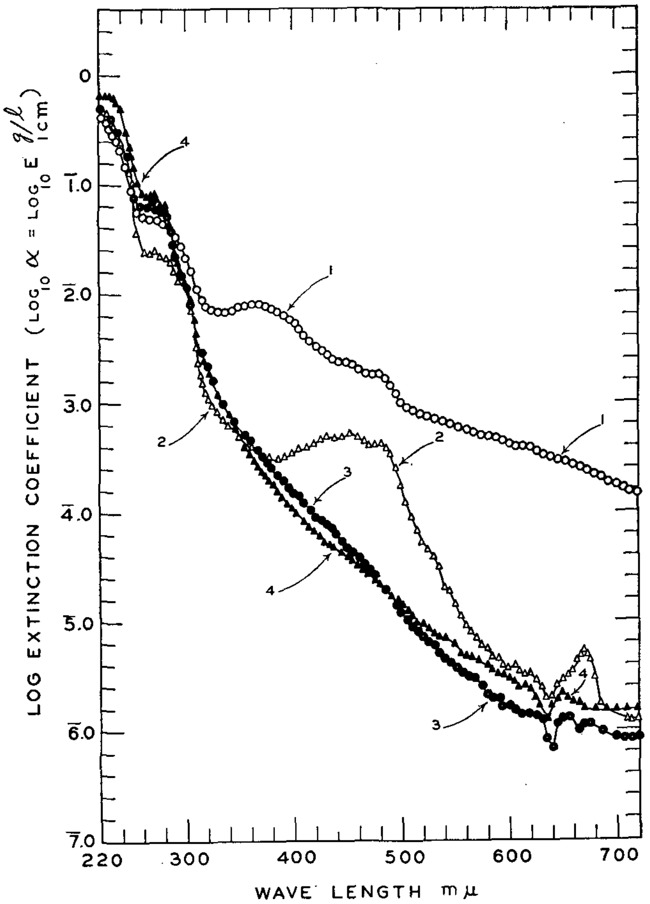

FIG. 1a. Spectra of cold hydraulic-pressed cottonseed oil containing 0.8% free fatty acid.

1. Crude.
2. Alkali-refined.
3. Alkali-refined and bleached.
4. Alkali-refined, bleached, and deodorized.

tion by gossypol is negligible. Increase in magnitude during refining and bleaching may be interpreted as due to isomerization during the alkali treatment and during adsorption and deadsorption accompanying the bleaching. This increase in conjugation during refining and bleaching is completely hidden in the spectra of the commercial hydraulic-pressed oils (Figures 1, 2, and 3) because of the greater overall effect of removal of the gossypol pigments. In all oil samples there is a marked increase in absorption at 232 mμ during deodorizing. As most gossypol pigments have been removed prior to this processing step, there is nothing to mask the increased conjugation produced at the high temperature involved in deodorizing.

In the spectra of all oils examined, the band at 268 mμ decreases in magnitude during refining and increases during bleaching and deodorizing. These effects are caused by a) the removal of gossypol during refining, the gossypol contributing to absorption at 268 mμ even in cold-pressed oils; b) isomerization during the adsorption and deadsorption accompanying the bleaching process; and c) oxidation or, more likely, dehydration, decomposition, or isomerization of oxidation products producing further conjugation at the high temperatures of the deodorization process. The intensities of the bands in the spectra of the commercial hydraulic-pressed oils are greater than in those for the cold-pressed hydraulic oils. This is due to the heating effect of the commercial process or to the greater gossypol content of the commercial samples or to both.

Sample number 5, the oil expressed by cold hydraulic pressing from seed stored for 58 months, exhibits a maximum at 232 mμ which is considerably more intense than found for sample number 4, the oil simi-

TABLE II

Some Extinction Coefficients × 10^3 of Crude Hydraulic Cottonseed Oils

No.	Seed processing	Type of processing	Wavelengths in mμ of significant absorptions					
			Diene 232	Triene 268	Gossypol 368	Carotene 450	Gossypurpurin 560	Pheophytin 670
1	Commercial	Hot	277	115	35.0	7.75	0.83	0.19
1a	Pilot plant	Cold	303	48.6	7.62	2.31	0.56	0.25
2	Commercial	Hot	336	165	46.6	12.4	1.73	0.55
2a	Pilot plant	Cold	302	86.0	23.8	5.97	1.62	0.77
3	Commercial	Hot	431	226	62.3	18.6	3.28	1.16
4	Pilot plant	Cold	1553	121	3.84	1.21	0.10	0.05
5	Pilot plant	Cold	2191	273	8.32	2.33	0.48	0.18

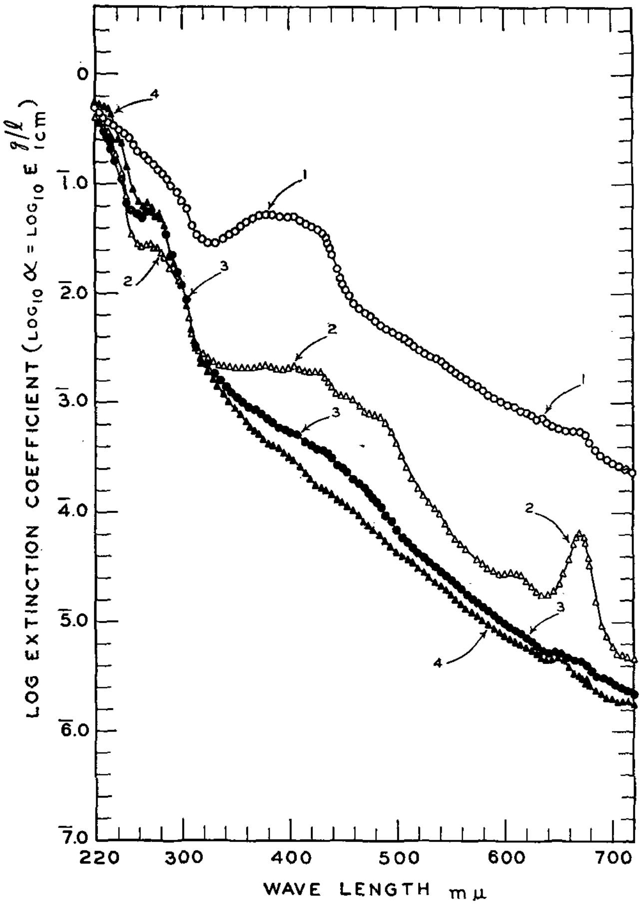

FIG. 2. Spectra of commercial hydraulic cottonseed oil containing 6.4% free fatty acid.

1. Crude.
2. Alkali-refined.
3. Alkali-refined and bleached.
4. Alkali-refined, bleached, and deodorized.

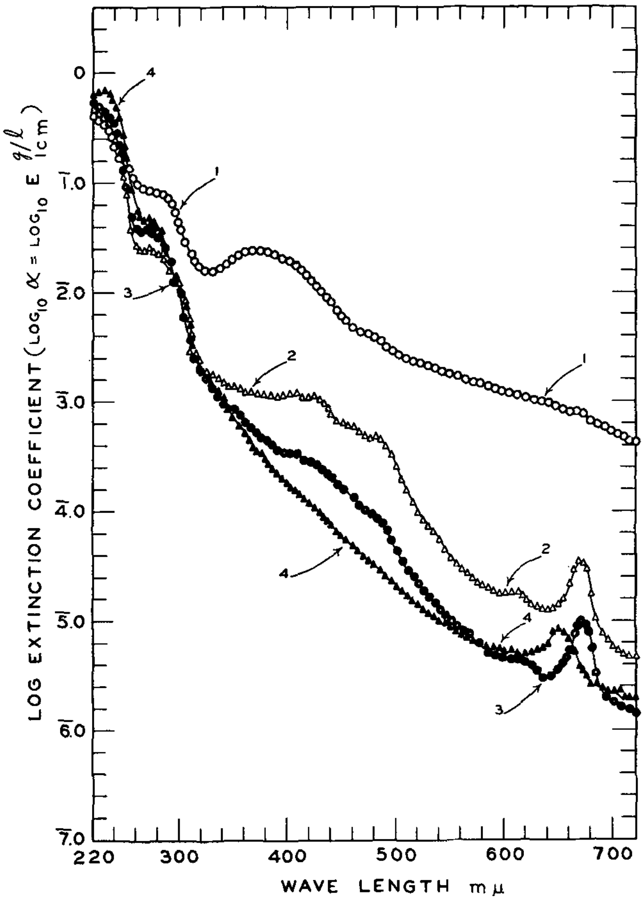

FIG. 2a. Spectra of cold hydraulic-pressed cottonseed oil containing 2.8% free fatty acid.

1. Crude.
2. Alkali-refined.
3. Alkali-refined and bleached.
4. Alkali-refined, bleached, and deodorized.

larly expressed from seed stored 15 months. This increased absorption during storage is due undoubtedly to increased diene conjugation resulting from oxidation during storage.

Visible Absorption. The near ultraviolet and the visible regions of the spectra can, for convenience of discussion, be divided into four absorption regions, each centered about a position of maximum absorption of one of the principal pigments of cottonseed:

Region	Center	Principal "Chromophore"
320 mμ-420 mμ	360 mμ	Gossypol
420 mμ-480 mμ	450 mμ	Carotene
480 mμ-640 mμ	560 mμ	Gossypurpurin
640 mμ-700 mμ	670 mμ	Pheophytin

In the near ultraviolet region of the spectra from 320 to 420 mμ absorption is due to gossypol and gossypol-like pigments. The crude hot hydraulic-pressed oils (Figures 1, 2, and 3) exhibit bands with maxima at 382 and 400 mμ and no indication of a band at 368 mμ, the position of maximum of gossypol absorption in oil (3). This has been interpreted as evidence that crude cottonseed oil, produced by modern commercial hydraulic press methods, contains very little, if any, gossypol, but does contain two gossypol-like pigments with maxima at 383 and 400 mμ (2). The cold hydraulic-pressed oils (Figures 1a, 2a, 4, and 5) exhibit absorptions with maxima at 368 to 374 mμ. This indicates the presence of some gossypol, maximum 368 mμ, and a third gossypol-like pigment, maximum 374 mμ. This latter pigment is identical to that observed in the spectra of screw-pressed and commercial hexane-extracted cottonseed oils (1). As these cold-pressed hydraulic oils were obtained from the same lot of seed as the commercial hydraulic-pressed samples, it would appear that there has been a modification of the pigments from gossypol with maximum at 368 mμ and a gossypol-like pigment with maximum at 374 mμ to two other gossypol-like pigments with maxima at 382 and 400 mμ and furthermore this modification of the pigments appears to be an effect of heat in the commercial hydraulic extraction process.

Refining removes the absorption in this region almost quantitatively. The fact that all of the maxima from 367 to 400 mμ are so completely removed is in itself an argument that these bands all arise from very similar pigments. Bleaching further decreases the absorption of all samples in this region, but this may be due to removal of background absorption from the carotene and carotenoid pigments absorbing maximally at longer wavelengths, which are removed by this bleaching process. Deodorizing again reduces the magnitude of the absorption throughout this region, probably by removal by volatilization of oxidation products which contribute to the general absorption of this region.

In the blue region of the spectra from 420 to 480 mμ, the absorption is due to carotenes and other carotenoids. No characteristic absorption bands are observable in the spectra of the crude hydraulic-pressed commercial oils, but the alkali-refined samples exhibit maxima at approximately 432, 455, and 480 mμ. The removal of the absorption due to gossypol in the alkali-refining procedure permits the weaker bands of the carotenes and carotenoids to be observed. These changes account for the marked differences in appearance between the spectra of a crude and a refined cottonseed oil.

In the cold-pressed hydraulic oils from the pilot plant (Figures 1a, 2a, 4, and 5) the low gossypol content makes it possible to detect the carotene and other carotenoid absorption bands in the crude oil. Removal of the gossypol pigment by refining however makes these bands more clearly visible. Thus, for all oils studied, there is evidence that alkali refining does not affect carotenoid content.

Bleaching completely removes all the absorption which is characteristic of carotenes and other carotenoids, and deodorization further reduces the absorption, probably by removing non-characteristic absorption oxidation products by volatilization.

In the spectral region between 480 to 640 mμ those pigments responsible for the red color of cottonseed oils exhibit characteristic absorption. Principal among these is gossypurpurin (1). However absorption bands characteristic of gossypurpurin at 535 and 560 mμ are usually not detected in cottonseed oils except those extracted from seed stored at elevated temperatures (4) even though many cottonseed oils have a decided dark red color. Obviously, other red pigments, as yet unidentified, occur in these cottonseed oils. In all the oil samples thus examined there are no characteristic absorption bands in this region. The absorption curves are very smooth from 500 to 600 mμ. This fact means that without preliminary concentration of the pigments, absorption spectra will not aid materially in the identification of the pigments responsible for the red color. It is an interesting observation, from the data presented in Table I, that the Lovibond color of the final processed, refined, bleached, and deodorized oil, with the exception of oil No. 5, the oil from seed stored for 58 months, follows in increasing intensity the order of the gossypol content of the original crude oil.

While no characteristic bands are observed in this region, all steps in the processing decrease the rather intense general absorption. The values in Table II show that this general absorption increases in intensity with the free fatty acid content of the oil and is greater in the commercial hydraulic-pressed samples than in the cold hydraulic-pressed samples of the same free fatty acid content.

Pheophytin with its most intense maximum at 670 mμ has been identified as the pigment responsible for the characteristic absorption in the region 640 to 700 mμ (2). This absorption is masked in the crude oils by the high background from the red pigments absorbing in the region below 640 mμ. Removal of these red pigments reveals the pheophytin band at 670 mμ

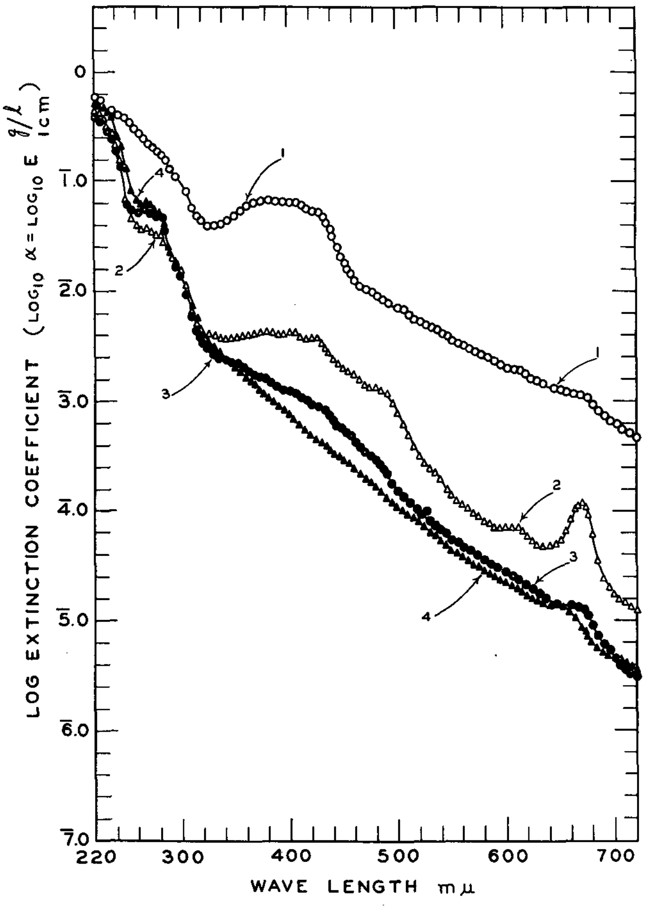

FIG. 3. Spectra of commercial hydraulic cottonseed oil containing 9.7% free fatty acid.

1. Crude.
2. Alkali-refined.
3. Alkali-refined and bleached.
4. Alkali-refined, bleached, and deodorized.

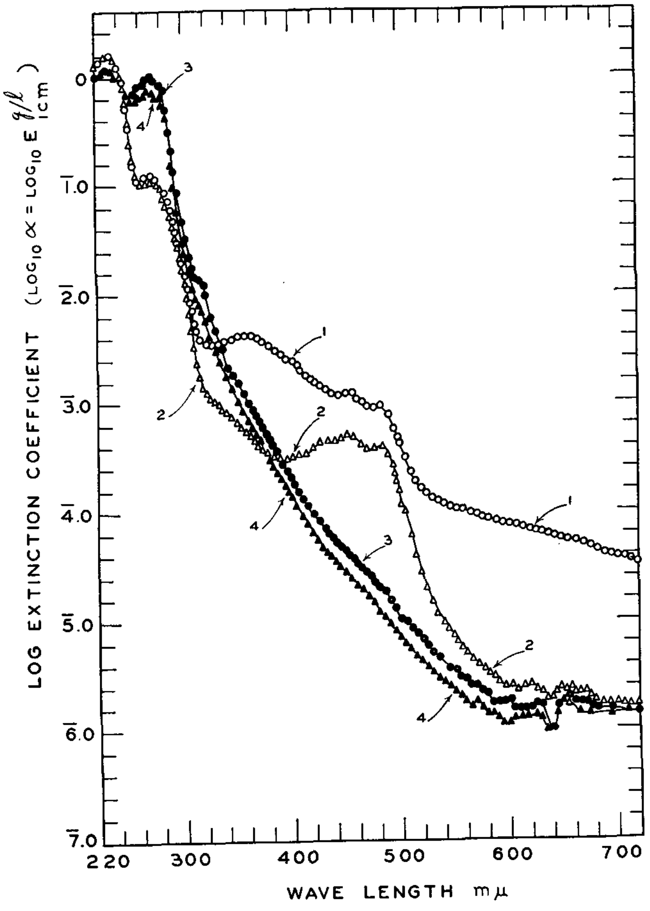

FIG. 4. Spectra of cold hydraulic-pressed cottonseed oil from seed stored 15 months.

1. Crude.
2. Alkali-refined.
3. Alkali-refined and bleached.
4. Alkali-refined, bleached, and deodorized.

as well as a less intense band of this pigment at 610 mμ. Bleaching completely removes the band, and deodorizing somewhat further decreases the absorption throughout this region. The intensity of the pheophytin bands in the crude oils increases with increase in free fatty acid content (Table II). This increase does not appear to be due to background absorption contributed from the red pigments, as this same order of intensity with increase in free fatty acid content at 670 mμ is observed in the spectra of the refined oils from which most of the red absorption has been removed.

Summary

Ultraviolet Absorption. The intensities of the bands at 232 and 268 mμ are invariably greater for the oil obtained by hot commercial hydraulic pressing than for the hydraulic cold-pressed oils from the same seed. This increased intensity is due to both the increased gossypol pigment content of the commercial hydraulic-pressed oil and to conjugation arising from oxidation during the heating accompanying this method of processing the seed. The intensities of these bands are also invariably greater in the spectra of the stored seed when compared to the spectra of the prime seed. This increased intensity is due to conjugation arising from oxidation during the storage period. The effect of refining and bleaching to produce increased diene and triene conjugation is masked in the spectra of the commercial hydraulic oils by the greater overall effect of the removal of gossypol-like pigments. If uncorrected, gossypol content can affect the accuracy of the determination of preformed diene and triene conjugation.

Absorption 320-420 mμ. The commercial hydraulic-pressed oils exhibit maxima at 383 and 400 mμ arising from gossypol-like pigments while the cold-pressed oils exhibit maxima at 368 mμ, the position of maximum for gossypol in oil and at 374 mμ, indicating a third gossypol-like pigment. As all these oils were from the same lots of seed, these differences appear to be due to the extraction process. As in the ultraviolet region the intensities of all the bands increase as the free fatty acid content of the oils increases.

Absorption 420-460 mμ. The crude hydraulic cold-pressed oils exhibit bands at 432, 455, and 480 mμ characteristic of the carotenes and other carotenoids. These bands are completely masked in the spectra of the crude hydraulic-pressed oils by the absorption due to gossypol. Refining accentuates the sharpness of these bands in the spectra of the pilot-plant cold-pressed oils and reveals them in the spectra of the commercial hydraulic-pressed oils. The intensity of the carotene and carotenoid bands increases with the free fatty acid content and is more intense in the spectra of the stored seed than in that of the prime seed.

Absorption 480-640 mμ. From a study of the spectra in the region between 480 and 640 mμ it can be concluded that the pigment responsible for the red color of cottonseed oils examined is not attributable to gossypurpurin, but to unidentified red pigments. Like the gossypol pigments, the intensities of these bands increase with the free fatty acid content and are considerably higher in the oil from the stored seed than in the spectra of the oil from the prime seed. These unknown red pigments are the principal cause of the dark red color of some cottonseed oils. Their identification is an important step toward a real understanding of the causes of dark red cottonseed oils.

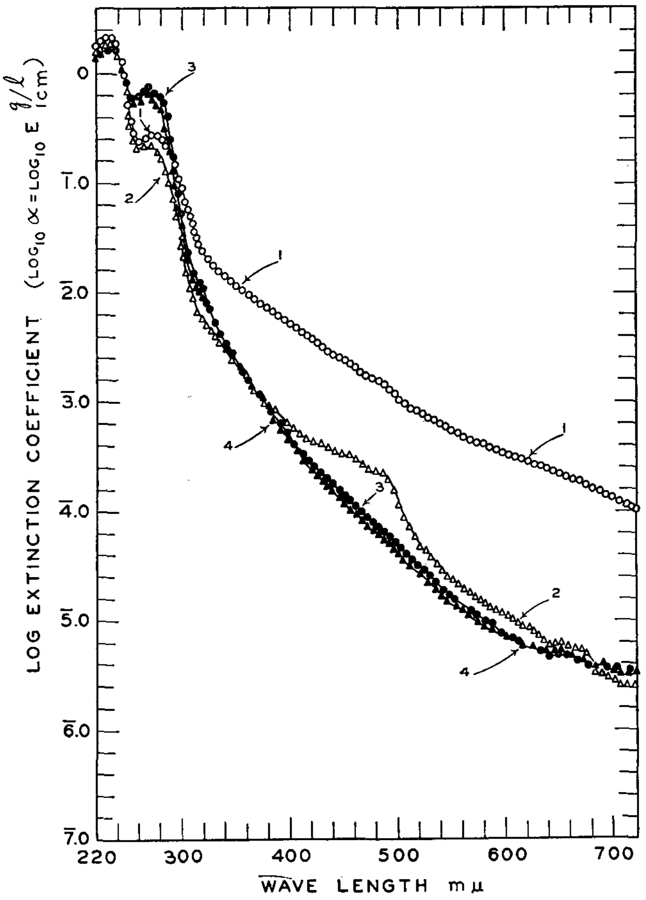

FIG. 5. Spectra of cold hydraulic-pressed cottonseed oil from seed stored 58 months.

1. Crude.
2. Alkali-refined.
3. Alkali-refined and bleached.
4. Alkali-refined, bleached, and deodorized.

Absorption 640-700 mμ. While the pheophytin is masked by background absorption of pigments in the region 480-640 mμ, after refining the characteristic band of this compound at 670 mμ accounts for the principal absorption in the region 640-700 mμ. In the refined oils the intensity of the pheophytin band increases with free fatty acid content and, as in these samples the absorption due to the red pigments has been largely removed, this increase in intensity cannot be attributed to increased background from this absorption.

Acknowledgments

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