Note on Gossypol and Its Relation to Color Fixation in Cottonseed Oil

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THE COLOR PROBLEM faced by the cottonseed oil industry has developed because of difficulty in removing fixed pigments from darkly colored oils by the current methods of alkali-refining and bleaching. The fixation of undesirable colors can develop during storage of crude oils obtained from prime cottonseed (12). It can also occur in the crude oils processed from immature, field-damaged, and storage-damaged mature cottonseed (2).

The inference from several observations is that the major coloration in cottonseed oil owes its origin to gossypol, the principal pigment of mature cottonseed. These observations are the following. a) The removal of acidic pigments, including gossypol, from freshly expressed prime crude oil by alkali-refining very soon after the oil is extracted, results in a product in which little pigment fixation has occurred. On the other hand, if the acidic materials are not removed immediately after oil expression, fixation of the pigments occurs, *i.e.*, pigments develop in the stored oil that cannot be removed by the conventional refining and bleaching procedures (2, 11, 12). b) Upon the addition of para-aminobenzoic acid to freshly extracted oil from prime cottonseed and the subsequent removal by filtration or centrifugation of the insoluble products formed, there then occurs a marked reduction in pigment fixation (3). c) There is reported a linear relationship between the initial content of total gossypol pigments and the increase in bleach color or fixed pigments which develops during oil storage (11). d) Oils from cottonseed meats cooked at low pH levels are high in total gossypol pigments and subject to red coloration during storage while those from meats cooked at high pH levels have a low refining loss, low total gossypol content, and are not subject to color fixation during storage (5).

Direct evidence is now presented that gossypol reacts with triglyceride oils to form yellow products which are not removed from the oils by alkali-refining. These are possible intermediates in the development of red coloration products which are also not removed by refining and bleaching.

Experimental

Determination of Gossypol. Although several spectrophotometric and gravimetric methods involving the use of aniline or para-anisidine have been developed for determination of total gossypol and gossypol-like pigments in cottonseed oil (1, 2, 4, 6–10), no distinction can be made between the actual gossypol content and that of gossypol-like pigments in an oil with the use of any one of these methods. For that reason it was necessary to employ a method whereby gossypol could be determined in the presence of any gossypol-like pigment formed during reaction of gossypol in oil. The method for gossypol determination reported in this paper was based upon an isolation procedure made possible by the acidic properties of gossypol, its water-insolubility, and its ability to form a water-soluble sodium salt. Since the distribution of gossypol between water and a water-immiscible organic solvent is influenced by the presence of alkali or acid in the aqueous phase, gossypol may be transferred from one to the other quantitatively by the addition of acid or base to the water phase.

The quantitativeness of the transfer was established for several water-immiscible solvents, including freshly refined, bleached, and deodorized cottonseed oil, isooctane, chloroform, and diethyl ether. In every instance all of the gossypol added to the organic phase was recovered by the extraction procedure indicated below.

Gossypol was extracted from the cottonseed oil, or other organic solvents, with a 0.5 N aqueous sodium hydroxide solution, which contained a small quantity of sodium dithionite. The aqueous phase was removed from the separatory funnel, acidified, and then immediately extracted with chloroform. The gossypol present in the chloroform phase was determined spectrophotometrically; the quotient of the optical density of the chloroform solution at 366 millimicrons and the extinction coefficient ($E^{1\%}_{1\,\text{cm.}} = 397$) for pure gossypol in chloroform at the same wavelength was multiplied by the proper dilution factors to give the concentration of gossypol in the original sample.

Color Determination. Oil colors were determined according to the American Oil Chemists' Society official Wesson and photometric methods (1).

Refining and Bleaching. The oil samples were refined and bleached according to methods recommended by the American Oil Chemists' Society (1).

Results

Examination of Crude Cottonseed Oils. The gossypol content of the crude commercial oils examined

TABLE I

Gossypol Contents and Colors of Commercially Processed Crude Cottonseed Oils

Description of crude oil[a]	Colors of oils			Gossypol content %
	A.O.C.S. photometric method	Lovibond colors[b]		
		Yellow	Red	
Straight hexane-extracted oil	96.3	70	22.0	0.07
High-speed screw-press oil	81.2	35	15.5	0.00
High-speed screw-press oil	77.9	70	17.4	0.00
High-speed screw-press oil	76.1	15	16.0	0.00
High-speed screw-press oil	74.0	20	14.0	0.00
High-speed screw-press oil	81.4	35	15.7	0.00
High-speed screw-press oil	78.8	35	17.0	0.00
Screw-press oil	42.0	35	8.6	0.00
Screw-press oil	40.6	20	8.5	0.00
Screw-press oil	48.0	35	7.0	0.00
Low-speed screw-press oil	81.6	15	17.0	0.00
Hydraulic-press oil	110.0	—[c]	—[c]	0.04
Hydraulic-press oil	102.0	—[c]	—[c]	0.01
Prepress (screw-press oil)	49.9	35	8.8	0.02
Prepress and solvent-extracted oil mixture	90.9	10	86.7	0.01

[a] Since samples were not analyzed immediately after expression from seed but were analyzed after receipt at SRRL, it was impossible to determine whether gossypol content of the oils underwent changes during that time interval.

[b] Colors determined with 1-in. columns of oils.

[c] Samples too dark for reading.

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was very low. From the data shown in Table I it can be seen that only traces, if any, of gossypol were recovered from crude screw-press oils and only very small quantities from the other oils.

Recovery of Gossypol Added to Organic Solvents. Actually the gossypol content of cottonseed oils is time-dependent since there was demonstrated a progressive disappearance of gossypol in these oils. For example, 260 milligrams of gossypol were added to 100 g. of a crude hydraulic-press oil; only 20 milligrams could be recovered from this oil one hour later.

This experiment was repeated with several solvents. Data are recorded in Table II. It is noteworthy that within one hour there was a loss of gossypol in each solvent which contained ester linkages, with one exception, namely, the loss of gossypol from a sample of freshly refined, bleached, and deodorized oil (*i.e.*, oil stored in the deep freeze away from air) was less rapid and could not be demonstrated after the lapse of only one hour. There was however a loss of gossypol even in this oil, but at a slower rate. On the other hand, the rate of gossypol disappearance was observed to be more rapid in a refined, bleached, and deodorized cottonseed oil (of commercial origin) that had been kept at room temperature and exposed to the atmosphere occasionally than it was in the corresponding sample of "fresh" cottonseed oil.

TABLE II

Gossypol Recovered After One Hour From Time of Its Addition to Various Samples

Description of sample	Gossypol present initially	Gossypol added	Total gossypol	Gossypol recovered by alkali
	%	%	%	%
Refined, bleached, deodorized oil (stored in deep freeze and not exposed to room atmosphere	0.002	0.090	0.092	0.092
Chloroform	0.000	0.120	0.120	0.120
Refined, bleached, deodorized oil (stored at room temperature and exposed to room atmosphere)	0.001	0.090	0.091	0.054
Crude screw-press oil	0.006	0.450	0.456	0.106
Crude hexane-extracted oil	0.067	0.140	0.207	0.111
Crude hydraulic-press oil	0.007	0.260	0.267	0.020
Tripelargonin	0.000	3.790	3.790	1.920
Ethyl acetate	0.000	0.088	0.088	0.050

Those esters, in which gossypol was lost, retained the yellow color on repeated extractions with 0.5 N aqueous sodium hydroxide solution. Obviously the yellow coloration products in these oils which are not extracted with the aqueous alkali are nonacidic derivatives of gossypol. It is interesting that the ultraviolet absorption spectrum in the region of 325–400 millimicrons of the yellow nonacidic derivative is very similar to that of gossypol.

One possible explanation for these observations is that the initial reaction between gossypol and the oils is that of an ester exchange, possibly one wherein gossypol plus triglycerides yield diglycerides and gossypol-fatty acid esters which are very much less acidic than gossypol. This seems reasonable since it is not expected that the esterification of one or two of the OH groups in gossypol would induce a significant shift in the ultraviolet spectrum, as the conjugation in the molecule would be the same as in gossypol. The formation of such esters should not efface the yellow color which is characteristic of gossypol.

The suggestion that the reaction is catalyzed by minor constituents in cottonseed oil are nullified by the observations that gossypol also disappears relatively rapidly in synthetic tripelargonin of very high purity and in ethyl acetate.

Secondary reactions occur in cottonseed oil and in tripelargonin, in which gossypol was lost, to give rise to red coloration products which are not removed by alkali-refining and on bleaching. An indication of the rate of color formation is given in the data recorded in Table III. Gossypol was added to a refined, bleached, and deodorized cottonseed oil that had been stored in the deep freeze and not exposed to the atmosphere prior to addition of the gossypol; and portions were stored at 98°F. and —26°F. It will be noted that about 95.5% of the gossypol added to an oil which was then stored at —26°F. was recovered after 63 days, and only a slight increase of Lovibond red color occurred. There was, on the other hand, a 40% loss of gossypol in the oil that was stored at 98°F. for 63 days, and there was also a marked change in the red color of the oil.

TABLE III

Effect of Gossypol on Colors of Refined, Bleached and Deodorized Cottonseed Oil

Storage conditions	Treatment of oil	Gossypol content (original)	Gram of gossypol extracted by NaOH	Lovibond color	
				Yellow	Red
		%	%		
Original samples	None	0.003	0.002	5	1.1
	Gossypol added	0.555	0.550	35	2.4
Stored 63 days at —26°F.	None	0.003	0.001	5	1.2
	Gossypol added	0.545	0.520	20	1.9
Stored 63 days at 98°F.	None	0.002	0.0007	10	0.7
	Gossypol added	0.480	0.2890	15	21.0

Removal of Pigmentation Because of Added Gossypol. It is significant that the intensity of the red color was not reduced by the standard alkali-refining and bleaching procedures (Table IV). The Lovibond red color of the red oil before it was refined was essentially the same after it was refined and bleached. The reading obtained, using the American Oil Chemists' Society photometric method with the refined and bleached oil, was not greatly different in value from that recorded for the Lovibond red color by the Wesson method, and they also indicate no effect on color intensity by the refining and bleaching procedures.

The development of the red coloration is not limited to cottonseed oil since the tripelargonin, to which gossypol was added, also gave a significant Lovibond red color (Table IV).

TABLE IV

Effect of Gossypol on the Refined and Bleached Color of Triglyceride Oils

Sample	Lovibond color		Color by A.O.C.S. photometric method
	Yellow	Red	
A. Fresh, bleached, and deodorized cottonseed oil–control	5	1.1	—
B. *Sample A plus 555 milligrams of* gossypol per 100 g. of oil	35	2.4	—
C. Sample A stored for 63 days at 98°F	10	0.7	0.6
D. Sample C refined	—	—	0.6
E. Sample B stored for 63 days at 98°F	15	21.0	—
F. Sample E refined	—	—	18.7
G. Sample E refined and bleached	10	23.2	18.5
H. Tripelargonin control stored at 98°F. for 63 days	0[a]	0[a]	—
I. Tripelargonin plus gossypol and stored at 98°F. for 63 days	35[a]	3.0[a]	—

[a] Colors determined with 1-in. columns of samples.

Summary

1. The crude oils studied contained from 0.00 to 0.04% gossypol.
2. Much of the gossypol added to crude cottonseed oils disappeared in one hour.
3. The disappearance of gossypol from refined, bleached, and deodorized cottonseed oil, from highly purified tripelargonin, and from ethyl acetate is demonstrable after the lapse of one hour.
4. It is suggested that the initial reaction of gossypol in the oils is an ester exchange reaction.
5. It is further suggested that secondary reactions result in the production of a red coloration product that is not removable from the oil by the standard refining and bleaching methods.

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Alcoholic Extraction of Vegetable Oils. Part IV. Solubilities of Vegetable Oils in Aqueous 2-Propanol[1]

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IN ASIATIC COUNTRIES, such as India, China, and Japan, the prices of conventional petroleum solvents are abnormally high because of heavy import taxes hence there is considerable scope for the use of lower aliphatic alcohols, which are known to be solvents for oils. The Japanese have reported the use of ethanol as a solvent for the batch extraction of soybeans in a plant at Darien, Manchuria (5, 11). Beckel and coworkers developed a continuous non-distillation process, using ethanol as a solvent while Harris and coworkers found 2-propanol[2] an excellent solvent for extracting cottonseed (2–4, 6–9). Work is in progress regarding the feasibility of using ethanol as a solvent for extracting cottonseed (15).

The most important and fundamental data necessary in studying the suitability of the solvents for extracting oils are the solubility data of the oils in different concentrations of these solvents. Recently comprehensive data on the solubilities of vegetable oils in aqueous ethanol solutions have been reported (13, 14, 16). A literature search revealed that while some data have been published on the miscibility of cottonseed oil in aqueous 2-propanol solutions, no information is available for other oils. Hence the need for this investigation.

Review of Previous Work

Harris and coworkers (6) have published data on the miscibility of cottonseed oil in 91% (by volume) 2-propanol, based on the cloud-point titration method. Magne and Skau (10) have reported the basic-phase, equilibrium data pertaining to the extraction of cottonseed oil with various concentrations of aqueous 2-propanol, based on work using the static, sealed-tube method. Thus data are available for cottonseed oil only and were obtained by the static method only, from which critical solution temperature *versus* 2-propanol composition data for use in design of the extraction equipment can be deduced by only an indirect method such as the one suggested by Okatomo (11). The aim of the present investigation was to obtain the basic solubility and critical solution temperature *versus* 2-propanol composition data by a direct method while also getting an idea of the working pressures developed by different concentrations of 2-propanol at various extraction temperatures.

Experimental

The apparatus (Figure 1) and the solubility determination method were the same as previously described (16). The various aqueous solutions of 2-propanol were obtained by diluting pure 2-propanol. The concentrations of the solutions were determined by the pycnometer method. All values are reported as weight percentage. Unrefined and commercially produced oils were used. Their characteristics are given in Table I.

TABLE I
Constants of Oils Used

Oil	Acid value	Iodine value (Wij's)	Saponification value
Babassu	2.8	12.8	248.6
Coconut	2.8	8.8	257.6
Corn[a]	1.5	120.2	189.7
Cottonseed	4.6	105.6	194.6
Linseed	1.5	182.5	191.3
Olive	1.1	84.6	190.4
Palm	3.4	53.9	199.6
Peanut	2.7	94.6	194.6
Rapeseed	2.7	105.6	171.6
Safflower	3.8	145.2	189.8
Sesame	2.5	112.7	191.4
Soybean	1.0	129.6	192.2
Sunflowerseed	1.7	123.8	190.8
Tung	1.5	168.7	192.5

[a] From wet-milled germs.

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[1] Presented at the 30th fall meeting, American Oil Chemists' Society, September 24–26, 1956, Chicago, Ill.

[2] Isopropanol or isopropyl alcohol.