Estimation of Gossypol in Crude Cottonseed Oil

By H. D. Royce

While it is well known that crude cold pressed or expeller oil contains gossypol, a review of the literature leaves the impression that crude hot pressed cottonseed oil does not contain this compound. Carruth[1] found no gossypol in oil samples pressed from properly cooked seed, and Clark[3] and Jamieson[5] both offer the opinion that hot pressed oil does not contain gossypol. Probably these conclusions were based on the fact that the Withers-Carruth[2] aniline method gave negative results when applied to hot pressed oil. Although Schwartze and Alsberg[7], and, very recently, Halvorsen and Smith[6] have developed improved modifications of the aniline method for the estimation of free gossypol in cottonseed meal, we have not found any published data on the estimation of gossypol in hot pressed oil.

The present paper describes an improvement of the aniline method which makes it possible to demonstrate that many hot pressed crudes contain gossypol in amounts up to 0.13%. While the presence of gossypol in crude cottonseed oil has little bearing on the quality of edible oil obtainable therefrom, since alkali refining removes gossypol quantitatively, it is significant in relation to refining losses and acid saponification of foots.*

The new method is based on the observation that pyridine promotes the precipitation of dianiline gossypol from oil solutions. The crude oil is diluted with petroleum ether and treated with a mixture of 4 parts pyridine to one part aniline, warmed, agitated, and allowed to stand for 3-6 days. After washing and drying, the precipitate is weighed. In some cases it is necessary to extract the dianiline gossypol from the amorphous insoluble sediment which occasionally contaminates the gossypol precipitate in samples of off oil or settlings. By the use of pyridine, crystalline precipitates or dianiline gossypol have been obtained from stock solutions containing as little as 10 milligrams of gossypol dissolved in 50 grams of refined cottonseed oil, from which no gossypol at all could be recovered by use of previous methods. The use of pyridine in the present method was suggested by a chance observation of a crystalline precipitate in a sample of crude oil which had been treated with a small volume of technical pyridine. The precipitate had much the same appearance as dianiline gossypol, and subsequent determinations of melting point, N content, and solubility showed it to be similar to the dianiline compound. Since the structure of the pyridine molecule precludes the possibility of a condensation analagous to the aniline reaction, it was suspected that the pyridine contained primary aromatic amines. A qualitative test for aniline was made on the technical grade, and its presence was confirmed.

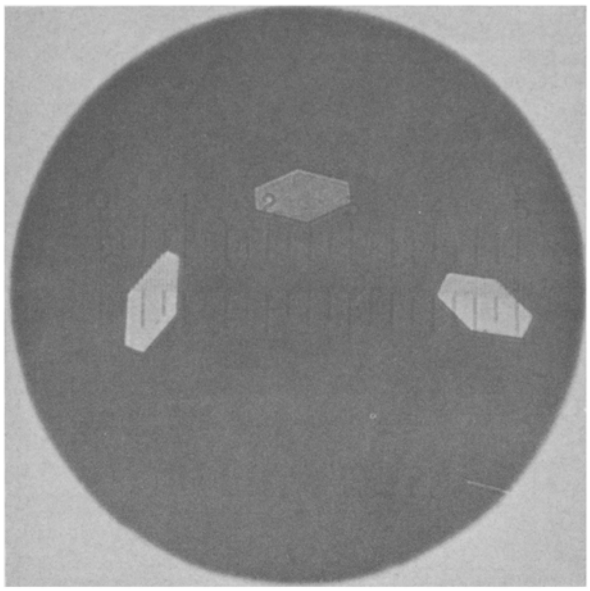

Fig. 1-a—Dianiline Gossypol-pptd by pyridine-aniline reagent cryst. from hot benyol

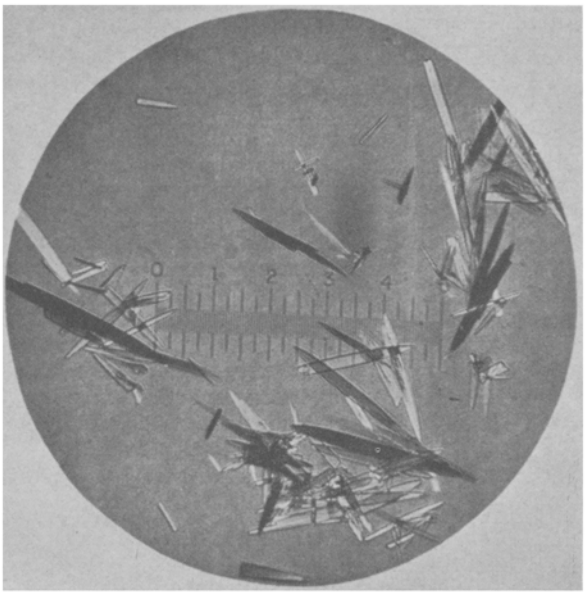

Fig. 1-b—Dianiline Gossypol-pptd by pure aniline crystallized from benyol

Although, as mentioned above, the gossypol compound precipitated from pyridine-aniline has a composition similar to the precipitate from aniline alone, the crystalline form is modified in the presence of pyridine (Figure 1-a, -b). This pronounced difference in crystal dimensions may explain in part why precipitation is more rapid and complete in the pyridine-aniline mixture.

Method in Detail

Fifty grams of crude (hot pressed) cottonseed oil are weighed into a 200 ml. extraction flask and diluted to 120-140 ml. with petroleum ether. If a precipitate forms at this time, or if the original oil contains sediment, filter on a suction plate and wash the paper with sufficient petroleum ether to replace evaporation loss and bring the final volume to about 140 ml. Add 12 ml. c.p. pyridine and 3 ml. aniline, warm the mixture slightly, and agitate on a mechanical shaker or stirring stand for 30-60 minutes. Stopper loosely to prevent excessive evaporation, and allow the samples to stand at room temperature for 3 to 6 days, or until precipitation has ceased. The dianiline gossypol usually precipitates as a crystalline brick-red powder which adheres to the walls of the flask. Filter on tared Gooch crucibles, wash with petroleum ether, dry 60 minutes at 100° C. and weigh. If the

*Royce and Lindsey, Ind. Eng. Chem., in press.

crystalline precipitate is contaminated with amorphous sediment which sometimes forms in off grade crudes, the dianiline gossypol must be dissolved through the filter, and the weight of the residue subtracted from the first weight. This is accomplished by pouring hot benzol or $CHCl_3$ over the precipitate until it runs through colorless. The crucibles are then dried and re-weighed. The difference in weights is recorded as dianiline gossypol, and this percentage, multiplied by the factor 0.775, gives percent of gossypol.[3] In removing the precipitate of dianiline gossypol from the extraction flask, it is generally necessary to use a rubber tipped rod, on account of the tendency of the crystals to "grow" on the walls of the container. Excessive washing is to be avoided due to the appreciable solubility of dianiline gossypol in petroleum ether. Drying the precipitate to constant weight is not a difficult matter, though it should be borne in mind that prolonged heating at temperatures much above 100° causes decomposition of dianiline gossypol.

Application of the Pyridine-Aniline Method

The accuracy of the pyridine-aniline method for the quantitative estimation of gossypol dissolved in oil was determined on a stock solution prepared by dissolving purified gossypol in Wesson Oil. Crude gossypol was extracted from choice seed and purified according to Clark's[4] method. The product was a yellow crystalline powder, m.p. 205° (uncorr.), mol. wt. 530 (titration with standard alkali), N in the dianiline derivative 4.28% (theoret. 4.19%). The stock solution was prepared by dissolving pure gossypol in ether, adding this to the Wesson Oil, and then evaporating the ether at low pressure. The gossypol concentration in the oil was adjusted to exactly 0.1%, so that a 50-gram sample contained 50 mg. gossypol.

TABLE I

Recovery of Gossypol from Stock (0.1%) Oil Solution. Comparison of Pyridine-Aniline with Other Methods, Using 50 Gram Samples Containing 50 mg. Pure Gossypol, Diluted with 75 ml. Petroleum Ether

Method	Pptg. Agent	Wt. Dianiline Gossypol	Wt. Gossypol (Factor .775)	Per Cent Recovery
1. Pyridine-Aniline (A)	Pyridine-Aniline 12 ml 3 ml	0.0616	0.0477	95
2. Pyridine-Aniline	12 ml 3 ml	0.0590	0.0540	90
3. Pyridine-Aniline	12 ml 3 ml	0.0602	0.0469	92
4. Carruth (B)	Aniline 3 ml	no. ppt.	.....	..
5. Halvorsen-Smith (C)	Aniline 4 Glycol 10	0.0181	0.0139	28
6. Halvorsen-Smith	.....	0.0113	0.0081	16

Analyses 1-6 (Table I) were all conducted in the same way, following identical conditions of agitation, heat, amount of solvent, and time, so that direct comparisons may be made. Only the pyridine method appears capable of precipitating gossypol quantitatively from oil solutions containing as little as 0.1%. These samples were allowed to stand 6 days before filtering. It is possible that longer standing might show some precipitation in the case of the straight aniline method, but from a practical standpoint such a procedure would not be desirable as an analytical method on account of the time factor.

From a total of 50 mg. gossypol present in a 50-gram sample, it will be seen that method A recovers an average of 46 mg., leaving but 4 mg., or a 0.008% solution in oil. Method C gives an average recovery of 22% in 6 days, which agrees with the recovery of 20% reported by Halvorsen and Smith[6] from a 0.1% solution, obtained by standing 8 days (5 mg. gossypol in 5 ml. oil).

TABLE II

Precipitation of Gossypol from Hot Pressed Cottonseed Oil (50 g. Sample, 75 ml. Petroleum Ether Added, Stood 6 Days)

Method	Oil	Wt. Ppt.	Wt. Gossypol	Per Cent Gossypol
A—(Pyridine 12-Aniline 3)				
Darlington	13	0.0676	0.0524	0.105
Bennettsville	11	0.0612	0.0471	0.091
Hartsville	176	0.0464	0.0349	0.070
Cordele	240	0.0320	0.0248	0.049
Birmingham	123	0.062	0.0470	0.094
Savannah	133	0.0560	0.0430	0.086
C—(Glycol 10-Aniline 4)				
Savannah	133	0.014	0.010	0.02
Birmingham	123	None	.....	....
B—(Aniline 3)				
Savannah	133	None	.....	....
Darlington	13	None	.....	....

The crude oils listed in Table II were selected at random from stocks on hand, and are mostly prime oils with low F.F.A. (0.5%-to 2.5%) and low refining loss. The superiority of method A is again apparent from inspection of the table. On the basis of these results it may be stated with certainty that the majority of hot pressed cottonseed oils contain gossypol.

In working out the best conditions for precipitating gossypol from oil with pyridine-aniline mixtures, it was observed that samples which had been heated to 100-130° C. gave low results. Several samples of crude oil were then heated alone prior to the addition of the reagents, and again results were low. This indicated that moderate heating renders gossypol non-precipitable. To check this point, 50-gram samples of a stock solution (0.5% pure gossypol added to crude oil) were heated to various temperatures, prior to analysis, and the results are summarized in Table III.

TABLE III

Effect of Heat on the Precipitability of Gossypol from Cottonseed Oil

(Period of heating, 30 minutes,—analyzed by method A)

No.	Temp. Deg. C.	Wt. Ppt.	Wt. Gossypol	Per Cent Gossypol
1	80	0.404	0.313	0.626
2	100	0.386	0.299	0.598
3	120	0.298	0.231	0.462
4	140	0.168	0.130	0.260
5	160	No ppt.	.....	.....
6	180	No ppt.	.....	.....

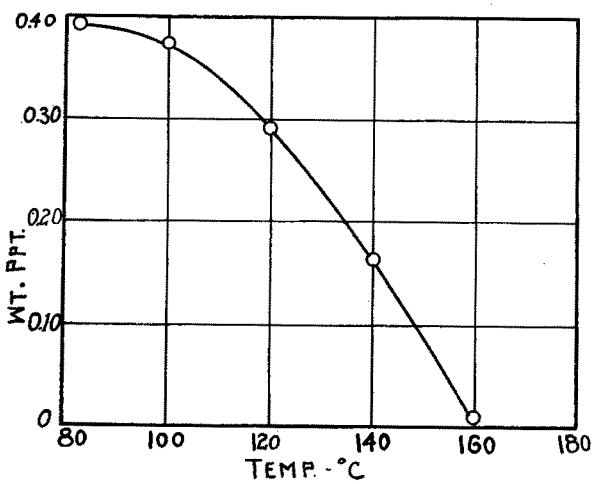

Fig. 2—Effect of a heat treatment on the subsequent precipitation of Gossypol from C. S. oil by pyridine-aniline (Gossypol solution (0.5%) in oil held at indicated temperatures for 30 minutes, then cooled and analyzed)

In the sample heated to 80°, precipitation was quantitative, 0.63% representing the added 0.5% plus 0.13% originally contained in the crude oil. After heating to 100° for 30 minutes, the yield was reduced appreciably,

and after heating to 160° no precipitation occurred. Figure 2 presents this data graphically.

Thus, while the pyridine-aniline method will precipitate gossypol from hot pressed oils which give negative results with former procedures, there is still a possibility that many crudes contain a "heat-modified" or strongly bound gossypol which is non-precipitable. In support of this opinion, it has been found that some crudes which have the refining properties of high gossypol oils do not give a precipitate with pyridine and aniline; and this same type of oil can be simulated by heating an oil containing precipitable gossypol to 100-120° C. Consequently the pyridine-aniline method has proved useful in checking cooker and press condition with regard to overheating, moisture, and length of cook in the crude mill. Also the method has been tested tentatively for the estimation of free gossypol in meal with excellent success, comparing favorably with the Halvorsen-Smith[6] procedure for this purpose.

Summary

1. Pyridine promotes the precipitation of gossypol by aniline from oil solutions. A quantitative method for the estimation of gossypol in crude hot pressed cottonseed oil is outlined, which involves the use of a pyridine-aniline (4:1) reagent.

2. Samples of crude oils from six different mills analyzed from 0.049 to 0.105 per cent gossypol.

3. Gossypol in crude oils is rendered non-precipitable by heating the oil to 150° C. for 30 minutes. Heating at lower temperatures decreases the yield.

4. Photomicrographs are presented which show the crystal form of dianiline gossypol precipitated in the presence of pyridine.

This work was carried out under the direction of L. C. Haskell, to whom grateful acknowledgment is made. The writer is indebted to M. C. Kibler for assistance in conducting the analyses.

Literature Cited

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[7]Schwartze and Alsberg, J. Agr. Research **25**, p. 285 (1923).

Welcome to New Members

The Membership Committee of the American Oil Chemists' Society is endeavoring to contact every chemist who is a prospective member of our Society, and it is hoped that the response will be even greater than anticipated.

Your Committee would like to encourage every member of the Society to do his utmost to interest someone in the work of their organization. We believe that the Society can be helpful to the chemists of our industry and we know the Society needs the co-operation of every man of his profession interested in the welfare of the oil and soap industry:

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W. D. HUTCHINS, Chairman,
Membership Committee.

Announcements

1. THE SEVENTH FALL MEETING of the American Oil Chemists' Society will be held in the Florentine Room of the Congress Hotel on October 12 and 13, 1933. On account of the crowded conditions of the Chicago hotels, we suggest you make your reservations at once, preferably at our headquarters, the Congress Hotel.

2. REGISTRATION will start at 9 o'clock Thursday, October 12. There will be the usual nominal registration fee of $1.00 to take care of the incidental expense of the Convention.

3. INFORMAL LUNCHEONS. We expect to continue our practice of having our informal lunch-