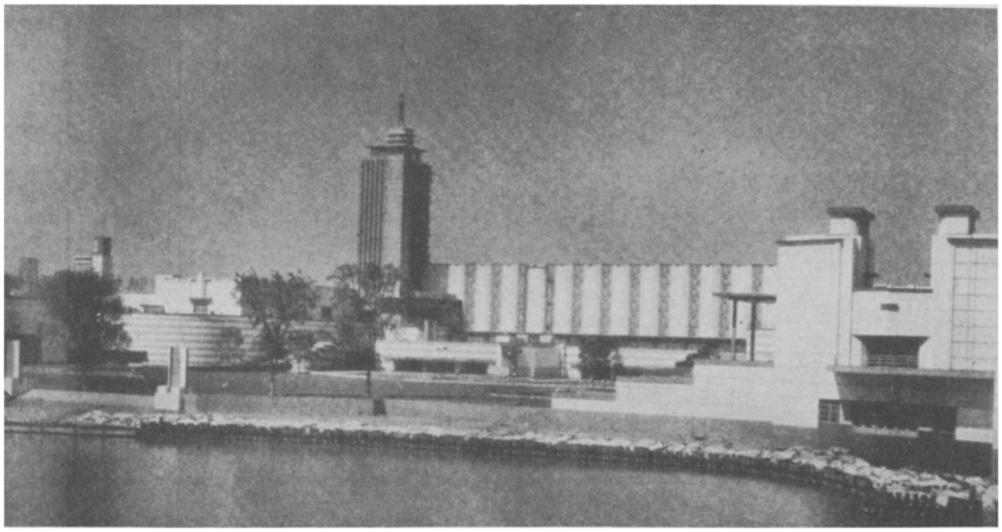

A FAMOUS VIEW AT CHICAGO'S CENTURY OF PROGRESS

GOSSYPOL

CONTENT AND REFINING LOSSES ON CRUDE COTTONSEED OIL

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A PAPER by the senior writer (3) published recently in this Journal describes a method for the quantitative estimation of gosspypol in hot pressed cottonseed oil. Prior to that time the common occurrence of gossypol in hot pressed oil was not generally recognized, since the older methods of analysis were not suitable for precipitation of small amounts of gossypol from oil solutions. In the original paper cited above, analyses of hot pressed oils from six southeastern mills showed gossypol percentages ranging from 0.049 to 0.105. During the past year gossypol determinations have been made on samples from the important cotton growing sections of the United States, and more than 90 per cent of all oils examined have contained appreciable quantities of gossypol. The maximum amount found in any hot pressed oil was 0.210 per cent, and the average percentage calculated from more than one hundred samples amounted to 0.058. While this figure is not of great magnitude compared with the average percentage of gossypol in cottonseed, reference to published data from this Laboratory (4) shows that the refining behavior of the crude oil may be affected by gossypol in such order of concentration, and that the antioxidant effect of gossypol is appreciable at these levels. Certain types of "high loss" crude oils have been found to respond well to a class of polyphenolic refining aids* soluble in an alkali refining solution, and gossypol falls in this classification.

For these reasons the refining losses on all of the oils which were analyzed for gossypol were tabulated, and deviations from the calculated average losses were plotted against gossypol content. Thus, while we have recognized in our previous work in this field that there are many factors besides gossypol concentration which influence the refining behavior of cottonseed oil, it was thought worthwhile to observe the gossypol-refining loss relationship while the data was at hand. The anomalous character of the results will be discussed in a later section of this paper.

Experimental

Some of the details of the original procedure (3) for the estimation of gossypol in hot pressed oil have been changed in the course of this work, and the modified method now in use is as follows:

The sample of crude oil (if tank car lots are being analyzed, obtain a representative portion by the official sampling method of the A. O. C. S.) is filtered on a fast paper (No. 230, Reeve-Angel). Weigh 50 grams of the filtered oil into a 200 ml. wide-mouth extraction flask and dilute the oil to 120 ml. with petroleum ether (35 to 60° C. boiling range). Add 15 ml. of the pyridine reagent, made up previously by dissolving 100 grams aniline in 400 grams purified pyridine (Eastman), and mix thoroughly. Stopper the flask loosely to retard evaporation and allow to stand at room temperature for 7 to 20 days, or until precipitation is complete. The time required for complete precipitation varies greatly with different types of crude oil, but in general samples containing over 0.02% gossypol precipitate completely in ten days. For oils containing less than 0.01% gossypol the precipitation is slow, sometimes requiring twenty days for completion. In analyzing these low gossypol oils it has been found advantageous to add a weighed amount (0.02%) of pure crystalline gossypol to facilitate crystal formation, later subtracting the calculated equivalent in dianiline gossypol from the weight of precipitate. After precipitation is complete, filter the dianiline gossypol on a tared Gooch crucible prepared with a light pad for rapid filtration. Usually a visible quantity of precipitate adheres to the walls of the precipitating flask, which must be loosened with a stirring rod. Rinse the flask with petroleum ether and wash the precipitate in the crucible

*U. S. Pat. app., Ser. No. 584,006 (1931), H. D. Royce.

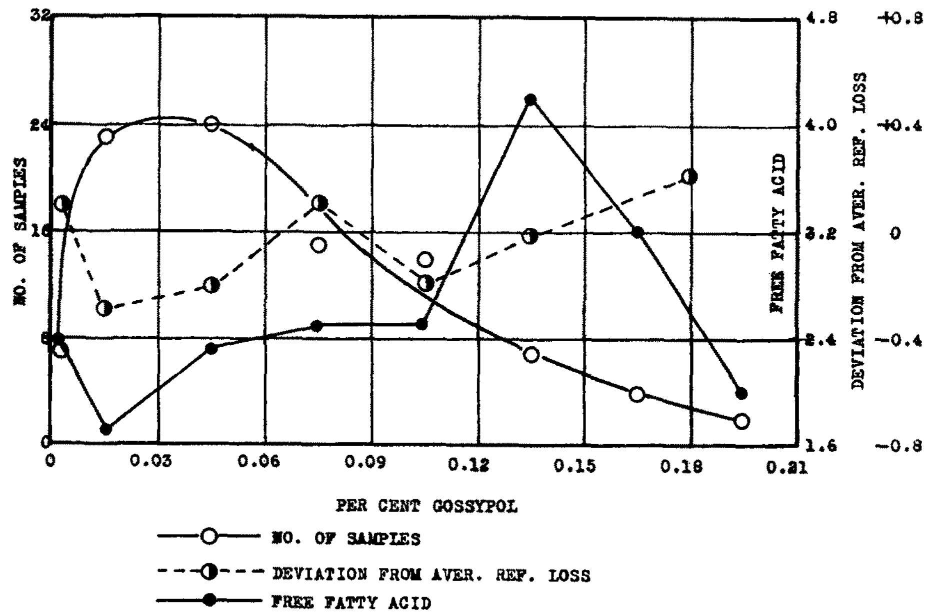

FIGURE I
Graphical analysis of data in Table I, showing variations in refining loss and F. F. A. averages plotted against Gossypol content for 96 representative crude cotton seed oils.

several times with this solvent until the dianiline gossypol is free from oil. Dry to constant weight at 100° C., and convert the weight of the dianiline gossypol to gossypol by use of the factor 0.775.

The above method has proven to be reasonably accurate and reliable on a wide variety of samples. Occasionally a crude oil will deposit a slight amount of amorphous sediment during the formation of the crystalline dianiline gossypol, and in such cases it is necessary to dissolve the dianiline gossypol through the filter with warm chloroform, substracting the weight of the residue from the total weight of precipitate initially recorded. If noticeable evaporation of the solvent in the precipitating flask occurs before the precipitation is complete, it is recommended that the sample be restored to its original volume by fresh addition of petroleum ether.

For application to quantitative free gossypol determinations in cottonseed or cottonseed meal, the above method needs but slight modification. The gossypol and oil are extracted by the method of Halverson and Smith (1), and the pyridine-aniline reagent is applied to the extract. Starting with a 75 gram charge of meal, the optimum amount of pyridine-aniline reagent is about 6 ml., substituted in the cited method in place of the ethylene glycol and aniline. If it is desired to determine the maximum amount of ether-extractable gossypol in cottonseed meal, the moist extractiton recently recommended by Halverson and Smith (2) may be used.

One hundred and twentyfour samples from 62 different mills were analyzed for gossypol by the above method. Only 7 samples failed to show the presence of gossypol, and individual determinations ranged from 0.001 to 0.210 per cent. The average of all analyses was 0.058 per cent. The samples were grouped in three divisions according to geographical location of mills. Group A comprises samples procured from mills in Georgia, the Carolinas, and Alabama; Group B from Tennessee, Arkansas, and Mississippi; Group C from Texas. A summary of results of gossypol content and refining data for oils of Group A is given in Figure 1. Free fatty acid and refining losses were determined by the official methods of the A. O. C. S. Average refining losses were calculated by means of the following equation, which was derived graphically from the observed F. F. A. values.

Average Refining Loss=2.3F.F.A.+4.25,

and "Deviation from average refining loss" plotted in Figure 1 represents the algebraic difference between the observed and the calculated average refining loss. For example, an oil is found to have F. F. A. of 1.0 per cent and loss 7.0 per cent. Then

Average Refining Loss=(2.3x1.0)+4.25=6.55, and Deviation from Aver. ref. loss=7.0—6.55=+0.45.

In plotting the curves of Figure 1, the samples were grouped according to gossypol content, averages being taken for each increment of 0.03 per cent gossypol. Thus, curve 1 is a distribution curve, showing a maximum between 0.03 and 0.06 per cent, which indicates that oils containing around 0.05 per cent gossypol occur more frequently than oils with higher or lower gossypol concentration.

Curve 2 represents the deviations from average refining loss over the whole range of gossypol content, and since the general trend of the curve is parallel to the X axis, it may be concluded that precipitable gossypol does not greatly influence the test refining loss. While this is in apparent contradiction to our results on the addition of free gossypol to alkali refining mixtures, the lack of agreement can be explained on two points—first, gossypol may be present in the crude oil in the free and/or the bound form. The bound form, on account of its association with nitrogenous protein fragments, probably does not have the same refining action as free gossypol. Secondly, it has been demonstrated that heating for a few minutes to 120° C. (3) renders free gossypol in oil nonprecipitable, and some of the crude oils in question may contain relatively large amounts of heat modified nonprecipitable gossypol which can affect the refining loss but is not precipitated by aniline.

Curve 3 shows that high F. F. A. oils generally carry more precipitable gossypol than oils of low F. F. A. Whether this fact has a real significance or not is doubtful, although it would be expected that damaged seed would give up gossypol freely to the oil on pressing on account of breakdown of the gossypol glands in the seed.

The following table summarizes the distribution of gossypol in crude oil according to localities, in Sections A, B and C:

TABLE I

Variations in Gossypol Content of Hot Pressed Crude Oils

	A Georgia, Carolinas, Alabama	*B* Tennessee, Arkansas, Mississippi	*C* Texas
No. of mills represented.....	36	14	12
No. of samples analyzed.....	96	16	12
Average per cent gossypol....	0.063	0.054	0.025
Maximum per cent gossypol..	0.210	0.128	0.082
Minimum per cent of gossypol	0	0.001	0.003

The above Texas oils have a definitely lower average gossypol content than oils from the southeastern seaboard, in line with the corresponding well known difference in gossypol content of the seed from these localities. However, it is not intended to draw general conclusions concerning gossypol in Texas oils until a larger number of samples have been analyzed over a period of several months or longer to allow for climatic and seasonal variations. The data on oils from the Southeast (A) are fairly complete for the first half of the 1933-34 season, and the average gossypol figures given herein represent a fair cross section of the production in that area. On account of the fact that the percentage of gossypol contained in hot pressed oil is but a fraction of the original percentage in the seed from which the oil was pressed, even in the high gossypol oils, the cooking and pressing conditions prevailing in the mill have a greater effect on the variations of gossypol in oil than the concentration of gossypol in the seed, since moderate heating in oil has been shown to affect its solubility (loc. cit.). Therefore it would not be surprising to find certain Texas mills, which operate on low gossypol seed, producing oil with a high gossypol content through the maintenance of cooking conditions favorable to the passage of gossypol into the oil. These observations, coupled with the fact that the percentage of precipitable gossypol in hot pressed oil does not by itself determine variations from average refining loss, lead us to conclude that gossypol exists in the oil in both the free and the bound form, and that the bound form does not function as a refining aid.

Acknowledgment

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Summary

1. Gossypol has been found to be almost universally present in hot pressed crude cottonseed oils from all of the important cotton growing sections in the United States.

2. The average amount found in crude oils from 62 scattered mills was 0.05 per cent. The highest percentage found in a hot pressed oil was 0.210. Only 7 out of 124 oils failed to show the presence of gossypol.

3. Since gossypol content was not found to be proportional to deviation from average refining loss, it was concluded that at least a portion of the gossypol occurs in the oil in the bound state, associated with protein fragments.

Literature Cited

[1]Halverson and Smith, Ind. Eng. Chem., Anal. Ed., *5,* 29 (1933).

[2]Ibid, *5,* 320 (1933).

[3]Royce, Oil and Soap, *10,* 183 (1933).

[4]Royce and Lindsey, Ind. Eng. Chem., *25,* 1047 (1933).