Removal of Gossypol From Cottonseed by Solvent Extraction Procedures[1,2]

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THE value of cottonseed meal as a feed depends not only on the nutritive value of the protein but also to a large extent on the presence or absence of materials which interfere with the growth of animals. Both of these factors are influenced by the method of processing of the cottonseed for oil and meal (1,7,12). The interfering materials have been shown to be concentrated in the pigment glands and to consist of gossypol and gossypol-like compounds (6,8,9). These materials may be removed from the meal by extraction with suitable solvents or by removal of the pigment glands with differential settling procedures; or they may be rendered inactive by binding them to the meal through cooking and screw press operations (2,3,10,11,14,15,18).

Boatner and co-workers demonstrated (3,4,5) the existence of gland walls enclosing the cottonseed pigments and also showed that these walls are readily ruptured on contact with water and certain organic solvents. Since gossypol and the gossypol-like pigments are more soluble in polar than in nonpolar solvents (3), a polar solvent-water pair should prove effective in extracting cottonseed pigments from the flakes. The research described in this paper was undertaken therefore, first, to determine on a laboratory scale the effectiveness of organic solvents and solvent-water pairs in lowering the gossypol content of cottonseed flakes. Then the solvent-water pair which proved to be the most effective extractant, namely, butanone (methyl ethyl ketone)-water, was used in experiments to determine the optimum conditions for the extraction of gossypol from cottonseed flakes. In the making of this study the effect of variations in the water content of the flakes and of the butanone and in the temperature of the extraction system on the rate and completeness of extraction was determined.

It is expected that the results of this investigation will be used in planning larger-scale production of meal and crude gossypol concentrates. Meals of low gossypol content are needed as a standard in cottonseed nutritional investigations that are being conducted by state and federal laboratories. The crude concentrate is needed for the preparation of pure gossypol and its derivatives for pharmacological investigations.

Solvents Used

The organic solvents, methanol, acetone, ethanol, isopropanol, butanone, dioxane, chloroform, 1,2-dichloroethane, trichloroethylene, and perchloroethylene were tested as well as 10 different solvent-water pairs. Diethyl ether, although it is recognized as an effective solvent for the extraction of gossypol from defatted flakes (3,6,13), was not included in the study since it was considered too hazardous to use in the equipment that was available for the quantities desired.

Analytical Procedures

Free and total gossypol. Free gossypol was determined by the method of Pons and Guthrie (16) and total gossypol by the method of Pons, Hoffpauir, and O'Connor (17). In these methods "free gossypol" is defined as that part of the gossypol and gossypol-like pigments extractable with aqueous acetone and "total gossypol" as that extractable by treatment with oxalic acid in aqueous methyl ethyl ketone under the conditions outlined in the methods for the determination of these products. "Bound gossypol" is total gossypol minus free gossypol. The quantity of gossypol that has been extracted is determined by measuring the amount of color produced when *p*-anisidine is added to the extract.

Moisture determinations. Moisture in solvent-free meal was determined by heating 5-g. samples of the meal in an air oven at 105°C. for 2 hours. Water in the butanone-water pairs was estimated from the specific gravity of the solutions. In the butanone-water meal extracts the Karl Fisher reagent modified for moisture determination in the presence of aldehydes and ketones was used (13). Experiments with solutions of pure gossypol, in concentrations ranging from 0 to 4 mg. per ml. of butanone and containing 5.1% water by weight, showed that the presence of gossypol did not interfere with the reagent during the determination of water.

Laboratory extraction procedures. The relative rates of extraction of gossypol from defatted cottonseed flakes by organic solvents and by organic solvent-water pairs were determined as follows: Twenty-five grams of flakes, previously extracted with hexane and containing 1.10% free gossypol, were soaked at 26°C. for 10 minutes in 100 ml. of solvent and stirred occasionally. The flakes were then freed from as much liquid as possible by suction on the Büchner funnel, were dried in the air, and the residual free gossypol was determined.

The effect on the rate of extraction of gossypol by butanone brought about by varying either the amount of moisture in the flakes or in the butanone and by varying the temperature of the extraction mixture was determined with the aid of a specially devised glass extraction cell. The cell consisted of a glass cylinder 4.8 cm. in diameter and 22.5 cm. in length. The bottom of the cylinder was closed with a coarse fritted-glass plate, and 17 cm. above the plate an outlet tube was sealed to the side of the cylinder. For the studies of the effect of temperature the cylinder was heated uniformly by electrical resistance ribbon, and the temperature was recorded from a thermometer inserted in the mass of flakes. Using 100-g. samples of flakes, 400 ml. of solvent were passed upward for 2⅔ hours, and free gossypol was determined on the

[1] Report of a study carried on under the Research and Marketing Act of 1946.

[2] This paper is No. 9 in the series on "Processing of Cottonseed" from the Southern Regional Research Laboratory. References to other papers in this series are: J. Am. Oil Chem. Soc. *24*, 97-108 (1947); J. Am. Oil Chem. Soc. *24*, 276-283 (1947); J. Am. Oil Chem. Soc. *24*, 362-369 (1947); J. Am. Oil Chem. Soc. *26*, 28-34 (1949); Oil Mill Gaz. *54* (2), 12-15 (1949); Cotton Gin and Oil Mill Press *51* (9), 18-20 (1950); Official Proc. Natl. Cottonseed Products Asso. *55*, 32-34, 36 (1951); and J. Am. Oil Chem. Soc. (in press), "The Effect of Screw Press and Hydraulic Press Processing Conditions on Pigment Glands of Cottonseed," by D. M. Batson, F. H. Thurber, and A. M. Altschul.

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dried extracted flakes as well as on successive 50-ml. portions of the percolate.

Results and Discussion

Effect of type of solvent. The relative rates of extraction of gossypol at 26°C. by pure organic solvents and by solvent-water pairs are given in Table I.

TABLE I

Effect of Solvents on Extraction of Free Gossypol From Defatted Flakes Containing Originally 1.10% Free Gossypol[1]

Solvent	Free gossypol in Extracted Flakes,%	Observations
Methanol	0.613	
90% aqueous methanol	0.789	
Acetone	0.710	
90% aqueous acetone	0.634	
95% aqueous ethanol	0.908	
90% aqueous ethanol	0.838	
Isopropanol	1.02	
90% aqueous isopropanol	0.90	
Butanone	0.76	
95% aqueous butanone	0.52	Flakes swelled slightly
90% aqueous butanone	0.225	Flakes swelled greatly
Dioxane	0.842	
90% aqueous dioxane	0.324	Flakes swelled greatly
Chloroform	0.874	
Chloroform saturated with water	0.898	
1,2-Dichloroethane	0.974	
1,2-Dichloroethane saturated with water	0.895	
Trichloroethylene	0.974	
Perchloroethylene	1.1 (approx.)	

[1]Extracted for 10 minutes at a temperature of 26°C. with 100 ml. of solvent used per 25 g. of flakes. Moisture content of flakes was 10.1%.

The butanone-water pair containing 10% of water was the most effective. It reduced the free gossypol in the flakes from 1.10% to 0.225% in 10 minutes. Dioxane containing 10% of water was nearly as effective; it reduced the free gossypol content to 0.324% in approximately the same length of time. Both the dioxane and the butanone caused an excessive swelling and packing of the flakes which made it difficult to remove the solvent from them.

The acetone-water pair containing 10% of water and the dry butanone were quite effective; the chlorine-substituted hydrocarbons were the least effective of the solvents.

Effect of water content of flakes and of butanone on extraction. The effect of the moisture content of the defatted flakes, 1 to 2% residual oil content, on the rate of extraction of gossypol with dry butanone is shown in Table II. Maximum rate of extraction was obtained when the flakes contained from 12 to 15% moisture. Changes in moisture content did not appreciably affect the percentage of bound gossypol in the extracted flakes. Swelling and packing of the flakes were observed when the moisture content was above 14%, which lowered the solvent percolation rate. This would indicate that either special equipment to diminish the packing effect or a lower moisture content would be essential when large masses of flakes are to be extracted.

TABLE II

Effect of Moisture Content of Defatted Flakes Containing 1.22% Free Gossypol, 1.32% Total Gossypol on the Removal of Gossypol by Dry Butanone[1]

Moisture in Flakes Before Extraction,%	Free Gossypol in Extracted Flakes,%	Total Gossypol in Extracted Flakes,%
6.8	0.816	0.90
8.4	0.533	0.59
8.7	0.435	0.55
12.6	0.098	0.20
14.7	0.120	0.21
14.7	0.123	0.22
15.3	0.051	0.19
17.5	0.133	0.24
21.1	0.154	0.24

[1]400 ml. of solvent at a temperature of 26°C. was passed through a column containing 100 g. of flakes during a period of 2⅔ hours.

Since 10% of water in the butanone was unsatisfactory, the effects of lesser amounts of water varying from 1 to 6% by volume were investigated, and the results are shown in Table III. The maximum rate of extraction was obtained when the solvent contained 5-6% of water by volume. The use of larger amounts of water in the solvent would probably increase the rate of extraction, but the resulting swelling and packing the flakes would then interfere with the flow of the solvent through the mass of flakes in a large extractor. Under such conditions there was a loss of water from the butanone and an increase in the moisture content of the flakes.

TABLE III

Effect of Water Content of Butanone on the Rate of Removal of Gossypol From Defatted Flakes Containing 5.7% Moisture, 1.21% Free Gossypol, 1.32% Total Gossypol[1]

Water in Butanone, by Volume, %	Water in Butanone, by Weight, %	Free Gossypol in Extracted Flakes, %	Total Gossypol in Extracted Flakes, %
0	0	0.855	0.92
1	1.22	0.631	0.68
2	2.44	0.185	0.35
3	3.65	0.209	0.30
4	4.88	0.094	0.24
5	6.10	0.105	0.21
6	7.32	0.029	0.18

[1]400 ml. of solvent at a temperature of 26°C. was passed through a column containing 100 g. of flakes during a period of 2⅔ hours.

Distribution of water and gossypol between butanone and cottonseed meal under equilibrium conditions. Under the conditions used to determine rate of extraction of gossypol it is unlikely that equilibrium conditions had been attained in the distribution of gossypol and moisture between the flakes and butanone. Experiments were therefore set up to determine this distribution under equilibrium conditions for meal of one moisture content.

The point of moisture equilibrium at 26°C. was determined by agitating for 2 hours 15-g. samples of hexane-extracted flakes containing originally 4.05% water with 150 ml. of solvent consisting of butanone containing known percentages of water by weight. The density of each butanone-water solvent and the corresponding extract was determined at the same time and at approximately 26°C.

The amounts of gossypol extracted and data for densities of the solvents and extracts are given in Table IV. The density of butanone containing small amounts of water was directly proportional to the water content. This relation was used in calculating the water content of the extracts from the density determinations. It was assumed that the contribution of the small amount of gossypol and oil in the extracts to the density was constant and of a low order of magnitude. The change in density therefore was considered to be due to changes in water content.

From the data in Table IV the percentage of water in the extracts corresponding to each solvent was calculated with the results illustrated in Figure 1. It is seen that a meal of 9.05% moisture content would be in equilibrium with an extract containing 2% water.

It must be recognized that age of meal and varia-

TABLE IV

Distribution of Water and Gossypol Between Butanone and Defatted Cottonseed Meal Under Equilibrium Conditions at 26°C.

Solvent Number	Water Content of Solvent by Weight, %	Density of Solvent, gm./ml.	Density of[1] Solution at Equilibrium, gm./ml.	Change in Density, gm./ml.	Water Content of Extract %	Gossypol Extracted, mg./ml.
0	0	0.7991	0.7993	+.0002	0.52	0.3015
1	1.244	0.8012	0.8015	+.0003	1.16	0.5600
2	2.480	0.8041	0.8040	—.0001	2.15	0.9895
3	3.670	0.8079	0.8061	—.0018	2.97	0.9410
4	4.892	0.8102	0.8086	—.0016	3.97	0.9105
5	6.125	0.8137	0.8110	—.0027	4.92	0.9540
6	7.335	0.8175	0.8135	—.0040	5.92	0.9410
7	8.487	0.8208	0.8154	—.0054	6.67	0.9085
8	9.739	0.8236	0.8167	—.0069	7.20	0.9540
9	10.897	0.8254	0.8185	—.0069	7.90	0.9540
10	12.115	0.8285	0.8191	—.0074	8.15	0.9150

[1]Density of solution after equilibration with cottonseed meal. The *effect of other solubles, such as gossypol and oil, on density is assumed* to be constant.

tion in conditions of removal of the oil with hexane may alter somewhat the conditions of equilibrium and the rates of extraction of gossypol. Pure butanone and butanone containing 1% water by weight gained water from the flakes. Equilibrium was attained between water in the butanone and in the flakes when the concentration of water in the solvent was between 1 and 2% by weight. At higher percentages of water in the butanone the flakes gained water in increasing amounts.

The amount of gossypol extracted under these experimental conditions increased with increasing water content in the butanone until a concentration of 2.5 weight percent was reached. Thereafter further increase in the water content of butanone did not increase the amount extracted.

Effect of temperature. The rate of extraction of gossypol by a water-butanone pair containing 4% water at temperatures ranging from 26° to 71°C. was determined with the results shown in Table V. Hexane-extracted flakes containing 1.21% free gossypol and 5.7% moisture were used in this experiment. The flakes extracted at 26° retained 0.08% free gossypol; those at 41°C. retained 0.072% free gossypol; and those at 71°C., 0.054%. That this decrease in residual free gossypol was due in part to the reaction of the gossypol with the protein in the flakes to form bound gossypol is suggested by the data in Table V, which indicate that the percentage of bound gossypol in flakes extracted at 26°C. was 0.11 whereas the bound gossypol in meal extracted at 71°C. was increased to 0.276%. The soluble nitrogen which is a measure of heat damage to protein was not appreciably affected in the range of temperatures used.

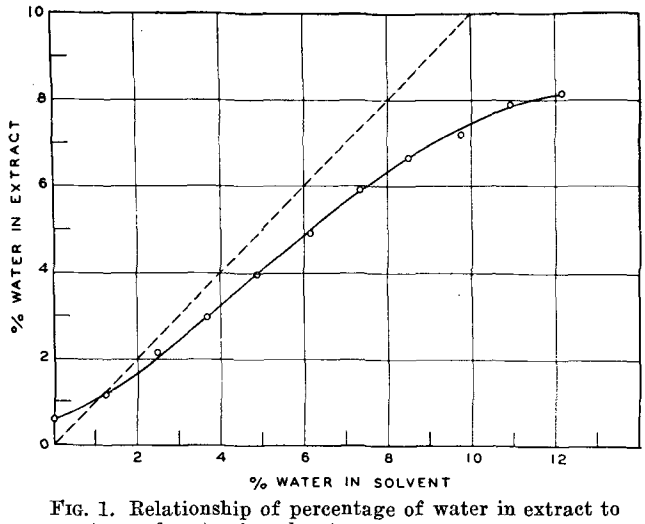

FIG. 1. Relationship of percentage of water in extract to percentage of water in solvent.

TABLE V

Effect of Temperature on the Extraction of Gossypol From Defatted Flakes Containing 1.21% Free Gossypol, 1.32% Total Gossypol, 5.7% Moisture, Using Butanone of 4% Water by Volume[1]

Temperature, °C.	Free Gossypol in Extracted Flakes,%	Total Gossypol in Extracted Flakes,%	Gossypol Bound,%	Total N Soluble in 0.5 M NaCl, %
26-27	0.08	0.19	0.11	77.8
41	0.072	0.16	0.088	77.6
51	0.062	0.18	0.118	73.2
60	0.052	0.23	0.178	71.6
71	0.054	0.33	0.276	70.4

[1]400 ml. of solvent was passed through a column containing 100 g. of flakes during a period of 2⅔ hours.

Summary

The relative efficiencies of organic, polar solvents and of solvent-water pairs for use in the extraction of gossypol and related compounds from cottonseed flakes were determined in a specially devised glass laboratory extractor.

Of the solvents tested a butanone-water pair containing 10% of water by volume was the most effective, and chlorine-substituted hydrocarbons were the least effective. Under equilibrium conditions maximum extraction of gossypol was obtained with a butanone solvent containing 2.5% of water by weight. The rate of extraction of gossypol from cottonseed meal with butanone-water pairs increased with increase in the amount of water in the system and with increase in temperature of the extraction system. The greater amounts of water in the extraction system resulted in swelling and packing of the flakes and in a decrease in extraction efficiency. Flakes extracted at 26°C. contained 0.08% free gossypol and those extracted at 71°, 0.054%. This decrease may be due, in part, to the reaction of gossypol with the protein to form bound gossypol.

REFERENCES

1. Altschul, A. M., Official Proc. Natl. Cottonseed Products Assoc., *55*, 32-34, 36 (1951).
2. Batson, D. M., Thurber, F. H., and Altschul, A. M., J. Am. Oil Chem. Soc. *28*, 468-472 (1951).
3. Boatner, C. H., Pigments of Cottonseed in "Cottonseed and Cottonseed Products," ed. by A. E. Bailey, Ch. VI, pp. 285-315, New York, Interscience, 1948.
4. Boatner, C. H., Hall, C. M., O'Connor, R. T., Castillon, L. E., and Curet, M. C., J. Am. Oil Chem. Soc., *24*, 276-283 (1947).
5. Boatner, C. H., and Hall, C. M., Oil & Soap, *23*, 123-128 (1946).
6. Boatner, C. H., Altschul, A. M., Irving, G. W. Jr., Pollard, E. F., and Schaefer, H. C., Poultry Sci., *27*, 315-328 (1948).
7. Dechary, J. M., and Altschul, A. M., Oil Mill Gaz., *54* (2), 13-15 (1949).
8. Eagle, E., Castillon, L. E., Hall, C. M., and Boatner, C. H., Arch. Biochem., *18*, 271-277 (1948).
9. Eagle, E., Arch. Biochem., *26*, 68-71 (1950).
10. Haddon, R., Schwartz, A. K., Williams, P. A., Thurber, F. H., Karon, M. L., Dechary, J., Guice, W., Kupperman, R., O'Connor, R. T., and Altschul, A. M., Cotton Gin and Oil Mill Press, *51*, (9), 18-20 (1950).
11. Harris, W. D., and Hayward, J. W., Texas Eng. Expt. Sta. Bull., *121*, 72 pp. (1950).
12. Lyman, C. M., Kuiken, K., and Hale, F., Texas Agr. Expt. Sta. Bull., *692*, 12 pp. (1947).
13. Mitchell, J. Jr., and Smith, D. M., "Aquametry," New York, Interscience, 1948.
14. Olcott, H. S., and Fontaine, T. D., Ind. Eng. Chem., *34*, 714-716 (1942).
15. Olcott, H. S., J. Am. Oil Chem. Soc., *25*, 125-127 (1948).
16. Pons, W. A. Jr., and Guthrie, J. D., J. Am. Oil Chem. Soc., *26*, 671-676 (1949).
17. Pons, W. A. Jr., Hoffpauir, C. L., and O'Connor, R. T., J. Am. Oil Chem. Soc., *27*, 390-393 (1950).
18. Vix, H. L. E., Spadaro, J. J., Murphey, C. H. Jr., Persell, R. M., Pollard, E. F., and Gastrock, E. A., J. Am. Oil Chem.. Soc., *26*, 526-530 (1949).

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