TABLE VI
Continuous Epoxidation of Soybean Oil[a]

Time (hr.)	Epoxy O (%)	Residual iodine no.
4	5.6	29
6	6.2	19
9	6.4	13
11	6.6	9
12	6.7	9
13	6.7	7
14	6.8	7
15	6.7	6

[a] Iodine No. of oil = 130. 1 mol oil/1.1 mol H_2O_2/0.5 mole acetic acid. 12% resin on oil. Temp. 60° C. in 1st 2 reactors, 70°C. in third. Approximately 80-minute hold-up in each reactor.

Table V shows the results from a typical experiment with methyl oleate, using 12% resin and an average reaction time of about 3 hrs., at 60°C. Equilibrium was reached at an epoxide conversion of about 90%. With 8% resin under these same conditions the product composition levelled out at a conversion of 81%. These results show that, as with batch reactions, about 12% resin should be used for the best results. Increasing the hold-up time however should produce results with 8% resin approaching those obtained with 12%. Economic operation will require re-use of the resin from this process. This can best be done by the partial replacement technique.

Use of a minimal quantity of resin (2% of oil weight) does not appear attractive for this process. With the temperature raised to 80°C. and a one-hour hold-up in each reactor, the conversion to epoxide was only 51%. As 58% of the unsaturation had been removed at this point, lengthening the time to increase conversion would lead to excessive by-product formation.

This process was also demonstrated with soybean oil. Using the same conditions as for methyl oleate with 12% resin, the product levelled off at 6.4% epoxy content and an iodine number of 14. To reduce the iodine number further the hold-up time for each reactor was increased to 80 min., and the temperature in the third reactor was raised to 70°C. As shown in Table VI, this raised the epoxy content of the product to 6.7% and reduced the iodine number to 1.

Summary

Commercial polystyrene sulfonic acid resins have been shown to be effective in catalyzing epoxidation of unsaturated fatty esters with hydrogen peroxide and acetic acid because they catalyze peracid formation but do not promote by-product formation when used under proper conditions. Special resins with less cross-linkage have been shown to yield mostly by-products.

Best catalyst life has been obtained with a special resin prepared with a low metal content.

A continuous process based on the cascade principle has been demonstrated in the laboratory. The problem of resin re-use for both continuous and batch processes can best be solved by using the optimum 10–15% based on oil weight and by replacing about 10% of the resin after each use.

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[Received October 23, 1957]

Binding of Gossypol Under Conditions of Complete Rupture of the Pigment Glands

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ATTENTION HAS BEEN FOCUSED on the "free" gossypol in cottonseed meals (as determined by the A.O.C.S. method) since it is felt by poultry investigators that very small quantities of this material in cottonseed meals fed to laying hens induce egg yolk discoloration (3). Previous work (5) has shown the presence in aqueous acetone extracts of cottonseed meal of materials in addition to gossypol, which yield colored reaction products when treated with aniline. Two properties distinguish these materials from gossypol, namely, a) they cannot be extracted from aqueous acetone with benzene, and b) the absorption spectra of the aniline reaction products differ from those of dianilinogossypol. Varying quantities of these materials are found in commercial cottonseed meals, and they may account for one-half to two-thirds of the aggregate of "gossypol and gossypol-like substances" as determined by the A.O.C.S. method for "free" gossypol (1).

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Raw cottonseed meats usually contain approximately 1.00% of "free" gossypol. A large proportion of the gossypol originally present in the seed is bound to the meal in the cooking operation. Most of the pigment glands are broken during rolling and cooking, and the liberated gossypol becomes bound under the influence of heat and moisture to meal constituents. The combined residual pigments (the "free" gossypol of the A.O.C.S. method), eluted by aqueous acetone in the analytical procedure, generally range from 0.02 to 0.06% in commercial meals, such as screw-pressed (2) and prepressed solvent-extracted meals, which are produced by processes involving attrition during the pressing operation. Hydraulic meals (2) usually contain approximately 0.10%, and uncooked, direct solvent-extracted meals 0.15% or more (8).

An effort has been directed toward finding a practical mechanical procedure for completely rupturing the glands so that all of the pigments can be bound to the meal in a subsequent cooking operation (6).

Results of that study indicate that it is not difficult to reduce the "free" gossypol content to the 0.02 to 0.06% range, but none of the methods studied resulted in further reduction.

It has been generally assumed, in the absence of evidence to the contrary, that the difficulty in achieving complete binding of gossypol has resulted from incomplete rupture of all of the pigment glands by any of the mechanical procedures used. The purpose of the experimental work in the present paper is to determine the fate of gossypol upon cooking cottonseed meats which have been subjected to complete gland rupture.

Experimental

Two procedures of gland rupture were used: a) a combination of drastic mechanical treatments, and b) a combination of mechanical treatment and the action of an aqueous polar solvent, known to facilitate rupture of the glands.

Mechanical Rupture of the Pigment Glands. Fresh, hulled cottonseed were flaked under high pressure in a rubber-working mill equipped with hardened steel rolls set at less than 0.0025-in. clearance. The rolls operated at different speeds, thus providing a shearing action in addition to the crushing action. This mill could not be fed moist meats so meats of normal moisture content (5 to 7%) were used. Moreover these meats had to be finely divided before they could be fed to the mill. This preliminary division was accomplished by first flaking to 0.012-in. thickness on smooth flaking rolls, followed by chopping in a conventional mechanical revolving-blade food chopper. Careful microscopic examination of the comminuted meats after passage twice through the rubber mill failed to reveal any intact glands. They were all mashed and almost completely denuded of meal tissue. This provided optimum conditions for contacting the glands with moisture during the subsequent operation. The meats were then placed in a planetary food mixer equipped with a flat beater and mixed at room temperature with sufficient water to bring the moisture content to 46% and with sufficient alkali (0.67% NaOH) to bring the final extracted meal to a pH value of 8. After one minute of stirring the slurry was extruded, by means of a gear pump, through a spring-loaded, standard ½-in. pressure relief valve. This device, being water-tight when closed, opened under pressure to an extent necessary to equalize the force exerted by the spring; thus it presented an extremely small opening around the periphery of the ground valve for the passage of the slurry of cottonseed meats. The object of this device was to force the passage of even the smallest glands through an opening sufficiently small to provide the shearing action necessary for their rupture while in a moist, weakened condition. A portion of this extruded material was dried in an oven at 105°C., then ground to 20 mesh in the Wiley[2] mill. Analysis for "free" gossypol by the A.O.C.S. method (1) and for gossypol by the benzene transfer method described below yielded values of 0.020% and 0.004%, respectively. No unbroken glands could be detected by careful microscopic examination of the meal.

These results led to the tentative conslusion that at least part of the residual "free" gossypol (as determined by the A.O.C.S. method) cannot be bound to the meal by the usual cooking procedures, and its presence is not caused by retention in unbroken pigment glands.

Chemical Rupture of the Pigment Glands. The above view was confirmed in a series of experiments wherein the raw meats were comminuted by the procedure used in the A.O.C.S. method for "free" gossypol, thus bringing the "free" gossypol of the aqueous acetone extract into intimate contact with the cottonseed proteins. A lot of cottonseed (variety Acala 440), from the 1956 California crop, was hulled and ground to 20 mesh in the Wiley mill. Portions of 0.5-g. each were suspended in 50.0 ml. of 70% aqueous acetone in 250-ml. Erlenmeyer flasks and agitated with glass beads for 1 hr., in accordance with the A.O.C.S. method for "free" gossypol. In each instance the acetone was boiled off on the steam bath, the sides of the flask were rinsed down quantitatively with distilled water, and the contents of the flask were taken to dryness on the steam bath at a final temperature of 98–100°C. A current of warm air was passed over the flask to speed the evaporation of the acetone and water. Approximately four hours were required in each instance to bring the contents of the flask to dryness. The flasks were then left in the steam bath, and heating was continued for the lengths of time shown in Table I.

TABLE I

Results of Analysis of Cooked Cottonseed Meats

Time of cooking	"Free gossypol and gossypol-like substances"[a]	Gossypol[b]	Gossypol-like pigments (by difference)	Total gossypol	
				by acid[c] hydrolysis	by hot aniline method
hours	%	%	%	%	%
0 (raw meats)	0.92	0.89	0.03	1.05	1.14
4	0.065	0.045	0.020	0.91	0.87
18	0.037	0.018	0.019	.....	0.80
25	0.024	0.011	0.013	0.82	0.77
30	0.012	0.003	0.009	.....	0.68
45	0.012	0.000	0.012	0.64	0.62

[a] By A.O.C.S. method for "free" gossypol.
[b] By extraction of aqueous acetone eluate (from [a]) with benzene.
[c] By A.O.C.S. method for total gossypol.

Results of Analysis of the Cooked Meats

Analytical Methods

"Free Gossypol and Gossypol-like Substances." Fifty ml. of 70% aqueous acetone were added to the flask, and the contents were agitated for 1 hr. "Free" gossypol was then determined spectrophotometrically, after heating an aliquot of the filtrate with aniline by the A.O.C.S. method. Results are given in column 2 of Table I.

While the "free gossypol and gossypol-like substances" reported in Table I are a measure of the aniline chromogenic material eluted with aqueous acetone, tests made on the replicate portions of the cooked meats showed that no aniline chromogenic material is eluted by benzene at ambient temperature, thus showing that this material is not dissolved in the oil. Apparently it is loosely adsorbed on the meal tissue though not completely bound as is the major portion of the hot aniline-soluble material.

Gossypol. The gossypol in another aliquot of the same aqueous acetone eluate was transferred to benzene by quantitative liquid-liquid extraction, and it was determined by spectrophotometric examination of the benzene solution after the benzene solution was

[2] Trade names are given as part of the exact experimental conditions and not as an endorsement of the products over those of other manufacturers.

heated with aniline (4). Results are given in column 3.

Total Gossypol (hot aniline method). Replicate 0.5-g. portions of the cooked meats were agitated in the flask with 25 ml. of aniline. The flask was then immersed in an oil bath heated to 200°C., and the aniline was allowed to boil until its vapors were emitted from the mouth of the flask (3 to 5 min.). After cooling, the entire contents of the flask were washed into a 500-ml. volumetric flask with benzene and adjusted to volume with the same solvent. A reagent blank was prepared by heating 25 ml. of the aniline in another flask. Sample blanks were prepared by agitating a replicate portion of the sample with 25 ml. of benzene. The dianilinogossypol formed by the reaction of aniline with the bound as well as the "free" gossypol in the cooked meats was determined spectrophotometrically (4) on portions of the filtered benzene solutions, and the total gossypol content was calculated after correction for absorbance of the reagent and sample blanks and the volume occupied by the glass beads used in preparing the sample. Results are given in column 6.

Total Gossypol (A.O.C.S. oxalic acid method). A replicate portion of the cooked meats was agitated with 15 ml. of the oxalic acid solution, the sides of the flask were washed down with the remaining 10 ml. of the required amount of the same solution, and the determination was completed by the A.O.C.S. method for total gossypol. Proper correction of the results obtained for volume of beads used in preparing the cooked meats was applied. Results are given in column 5.

"Free" Gossypol from Bound Gossypol. In order to determine if the "free" gossypol in the eluate is derived from the bound gossypol by partial elutriation of the bound gossypol, the residue from the "free" gossypol determination of a portion of the meats which had been cooked 18 hrs. was re-extracted with another 50-ml. portion of the aqueous acetone. No further "free" gossypol was removed.

Hydrolysis of "Gossypol-like Substances." An aliquot portion of the aqueous acetone eluate from meats cooked for 18 hrs. was heated at 65°C. for 1 hr. with 1% of HCl. Recovery of 0.036% of gossypol by benzene extraction showed that under these conditions the "gossypol-like substances" were converted to gossypol.

Discussion

Even on complete rupture of the pigment glands, complete binding of the gossypol to the meal by cooking is a very slow process; under the conditions studied 30–45 hours of cooking are required to reduce it to zero. All of the "gossypol-like substances," which are a part of the aggregate of pigments determined by the A.O.C.S. method for "free" gossypol, are not eliminated even on very prolonged cooking (up to 45 hrs.) at 100°C. It was found that these pigments may be converted into gossypol by heating the aqueous acetone eluate with HCl.

All of the pigments, including bound gossypol, are reduced by prolonged cooking. The good agreement in the values obtained for total gossypol (columns 5 and 6 in Table I) by two widely different chemical methods of analysis support the supposition that either method might measure all of the gossypol-like pigments in cottonseed meal.

Summary

The "free" gossypol of cooked cottonseed meats is composed of gossypol and of "gossypol-like pigments," which are soluble in 70% aqueous acetone. The "gossypol-like pigments" may account for 30 to 100% of the "free" gossypol as determined by the A.O.C.S. method.

Thorough comminution, followed by prolonged cooking, results in reduction of all the pigments in cottonseed meats. "Free" gossypol is not completely eliminated, but the gossypol level, as determined by the benzene transfer method, may be reduced to zero.

"Gossypol-like pigments" are converted into gossypol by the action of hot aqueous hydrochloric acid.

Good agreement is observed between the data for "total" gossypol, as determined through the use of the A.O.C.S. oxalic acid method and through the use of hot aniline.

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[Received December 19, 1957]

Report of Cellulose Yield Committee, 1957-58

THE FOLLOWING TABLE gives the averages of the lint yield analyses from three sets of samples sent out during 1957 and 1958. All three samples were second-cut linters.

Lab. No.	No. of tests	A Linter	B Linter	C Linter	Over-all avg. for the year
1	3	77.7	74.6	69.8	74.0
2	3	78.7	76.3	70.6	75.2
3	3	77.9	75.0	71.0	74.6
4	3	77.6	74.6	70.6	74.2
5	3	78.0	75.0	70.0	74.0
6	3	78.4	75.4	70.3	75.0
7	3	78.0	75.0	70.0	74.0
9	3	78.0	75.0	69.0	74.0
10	3	78.5	76.0	70.9	75.1
Average		78.0	75.2	70.2	74.4

The above analyses are very good; however some work is necessary as a couple of laboratories are a little on the low side. Work will be done during the coming year to bring all the laboratories more nearly in line. We recommend that samples be sent out three times during the next year to keep all equipment in good repair.

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