

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.

## REFERENCES

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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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