Table III, recalculated on an oil-free basis, are shown in Table IV and Figure 4. At low humidities the curve for the hygroscopic equilibrium of cottonseed (on an oil-free basis) is intermediate between the curves for flaxseed and sunflower seed. However, the moisture content of cottonseed increases more rapidly at humidities above 70% than does that of either flaxseed or sunflower seed. Furthermore, at 93% relative humidity cottonseed attains a higher equilibrium moisture content than do flaxseed and sunflower seed but one lower than that of soybeans.

TABLE III

Equilibrium Moisture Contents of Cottonseed, Sunflower Seed, Flaxseed, and Soybeans at Various Relative Humidities

Relative humidity	Moisture content (wet basis)			
	Cottonseed	Sunflower seed[a]	Flaxseed[a]	Soybeans[a]
%	%	%	%	%
31.0	6.0	5.2	5.3	6.1
43.0	7.2	6.3	6.4	7.4
51.0	......	6.9	6.9	8.3
62.0	9.3	8.1	8.2	10.4
71.2	10.3	9.5	9.5	12.4
81.1	13.2	11.7	11.6	16.4
93.0	22.2	16.9	17.1	25.1

[a] Data from Larmour, Sallans, and Craig (8).

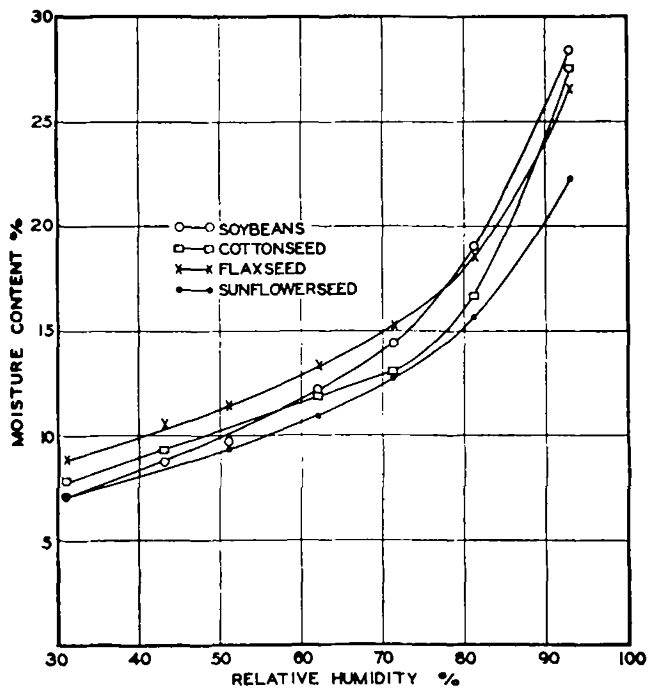

FIG. 4. Equilibrium moisture content at various relative humidities of soybeans, cottonseed, flaxseed, and sunflower seed on an oil-free basis. Data for soybeans, flaxseed, and sunflower seed from Larmour, Sallans, and Craig (8).

TABLE IV

Equilibrium Moisture Contents of Cottonseed, Sunflower Seed, Flaxseed and Soybeans at Various Relative Humidities Calculated on an Oil-Free Basis

Relative humidity	Moisture content (wet basis)			
	Cottonseed	Sunflower seed[a]	Flaxseed[a]	Soybeans[a]
%	%	%	%	%
31.0	7.8	7.1	8.9	7.1
43.0	9.3	8.6	10.6	8.7
51.0	......	9.3	11.4	9.7
62.0	12.0	10.9	13.3	12.2
71.2	13.1	12.7	15.2	14.4
81.1	16.7	15.6	18.6	19.0
93.0	27.5	22.0	26.3	28.5

[a] Data from Larmour, Sallans, and Craig (8).

Summary and Conclusion

An investigation has been made of the hygroscopic equilibrium of cottonseed over a range of 31% to 93% relative humidity. From 31% to 71% relative humidity the moisture content of cottonseed increased linearly from 6.03% to 10.27%. From 71% to 93% relative humidity the moisture content increased rapidly from 10.27% to 22.19%. When cottonseed was separated into meats and hulls, including linters, it was found that the hulls contained more moisture than the meats.

On the basis of these results it is apparent that, when stored cottonseed is aerated, consideration should be given to the effect of local atmospheric conditions. The relative humidity of the air used for aeration can affect the moisture content of the stored seed either favorably or adversely. Although it may temporarily reduce heating by conduction of the heat of respiration, it may increase the moisture content and thus stimulate further respiration and heating.

REFERENCES

1. M. L. Karon and A. M. Altschul, *Plant Physiol.*, *21*, 506-521 (1946).
2. Lillian Kyame and A. M. Altschul, *Plant Physiol.*, *21*, 550-561 (1946).
3. M. K. Thornton, Jr., and P. P. Briggs, *Oil Mill Gazetteer*, *33*, (6), 15-25 (1929).
4. M. K. Thornton, Jr., and F. E. Bishop, *Oil Mill Gazetteer*, *42*, (12), 11-15 (1938).
5. C. M. Franco, *Bragantia*, *3*, 137-149 (1943).
6. D. M. Simpson and P. R. Miller, *J. Am. Soc. Agron.*, *36*, 957-959 (1944).
7. H. M. Spencer in International Critical Tables, McGraw-Hill Book Company, Inc., New York & London 1926, Vol. 1, p. 67.
8. R. K. Larmour, H. R. Sallans, and B. M. Craig, *Can. Jour. Res.*, *22F*, 1-8, (1944).

Stability of Bound Gossypol to Digestion

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THE ether soluble, toxic gossypol of cottonseed is largely converted to the ether insoluble, "bound" gossypol in the cooking process. It is generally assumed to be bound to amino groups of the protein from analogy to the combination with aniline to form the very insoluble dianiline gossypol, which was found by Withers and Carruth (1) to be non-toxic to rats and rabbits. It colored the light colored feces of rats on a milk-starch diet a distinct orange color. Clark (2) found that "bound" gossypol in cottonseed meal could be converted to free gossypol after extraction with hot aniline.

Gallup (2) reported apparent digestibility coefficients of 61.2% to 71.5% for cottonseed meal protein compared to 73.6% to 81.2% for ether extracted raw cottonseed in rat diets. This suggests that the bound gossypol may have withheld some protein from digestion. Jones and Waterman (4) seeking to explain the relatively low digestibility of cottonseed meal protein, found that peptic and tryptic digestion of casein and

* The author is indebted to Dr. T. F. Zucker of Columbia University for the samples of feces and the "Proflo" flour and information that the feces weighed 28% of the diet.

cottonseed globulin was reduced 15% when treated with 1% gossypol. But since they evaporated a solution of gossypol on the dry proteins, at a temperature not exceeding 60° C., it is very doubtful that they duplicated the binding of gossypol such as occurs in the hot moist maceration of seed tissue in the cookers of the oil mill.

There is very little information on the fate of bound gossypol and of the protein to which it is assumed to be bound. Apparently the first quantitative study was with hens by Swensen, Fieger, and Upp (5) in connection with egg yolk discoloration by cottonseed meal. They recovered, in a control experiment without ferric chloride, 39.2% of 1.39 grams total gossypol ingested. They recovered 94% of the total gossypol ingested in a diet containing 0.5% ferric chloride and 30% cottonseed meal. The ferric chloride binds the gossypol presumably through the hydroxyl groups and prevents the discoloration of egg yolks.

After demonstrating qualitatively an abundance of gossypol in the feces of rats fed on a diet containing 60% cottonseed flour* the author asked Dr. J. O. Halverson and F. H. Smith of North Carolina State College for more accurate analyses by their two-gram method for gossypol (6).

The table below shows their analyses and shows that 86% of the bound gossypol in the diet was recovered in the feces:

	Free	Bound	Total
% Gossypol in flour	.1	1.03	1.13
% Gossypol in dry feces	....	....	1.9
Weight gossypol in diet	.06	.618	.678
Weight gossypol in feces	....	....	.532

The dry feces weighed 28% of the food intake and contained 31% ash and 6.3% nitrogen.

Absence of Gossypol in the Salt Soluble Protein of Flour

Slightly more than half of the nitrogen of cottonseed flour is soluble in 10% sodium chloride solution. The light color of the extract and the whiteness of the precipitated protein, shows that practically no gossypol is extracted. The salt extract turns a pale yellow color when made alkaline, but this color is stable and does not darken and subsequently fade as when gossypol is present.

The protein in the salt extract (200 c.c.) of 10 grams of flour was precipitated with dilute HCl and the whole mixture shaken with ether. The ether was separated and evaporated with a drop of aniline present. The color was only slightly more yellow than the control without aniline. A portion of the salt extracted flour, which was obtained in a powdery condition by washing with water, methanol, and hexane, was tested with aniline and ferric chloride. The tests indicated that by far the greatest part of the gossypol was retained here. (Analysis 1.74%, Halverson and Smith.)

This raises the question as to whether only certain proteins in the flour are combined with gossypol or whether other substances bind the gossypol. Extraction of the powdery residue with 90% formic acid and testing the fractions, showed the greatest part of the gossypol was in the soluble part. Evaporation of the formic acid left a protein film which dissolved in H_2SO_4 with a deep red color. The film was heated with aniline. On dilution of the aniline with benzene, there was a much greater intensification of color, characteristic of gossypol, than when the residue on the filter was given a similar treatment. It is thought that these qualitative tests show that formic acid, a good protein solvent, has dissolved the gossypol complex.

Combining Ratio of Gossypol and Protein

Assuming that gossypol, $C_{30}H_{30}O_8$, of molecular weight 518, combines with two amino groups through its two aldehyde groups, the ratio of gossypol to nitrogen is 518 to 28, or 1 to 0.054. In the cottonseed flour, the ratio of bound gossypol to nitrogen is approximately 1 to 9, and to protein 1 to 55.

On the above assumed combining ratio, the gossypol would be bound in the ratio of 0.054 to 9 for nitrogen or 0.33 to 55 for protein (1 to 166) which would mean only 0.6% of the nitrogen or protein.

Gossypol forms complexes with polar oxygen compounds such as acetic acid and acetone only in nonaqueous media, so far as known. Gossypol reacts with dilute aqueous ammonia to form a gelatinous mass. It is thought that under the hot moist conditions of cooking cottonseed, gossypol issuing from the glands in a very fine suspension, combines with certain polar amino-groups to form a complex resistant to digestion. It is suggested that an aqueous phase is involved, since under hot dry pressing, the bulk of the gossypol is dissolved by the oil and retained in it.

Summary

Bound gossypol is largely unabsorbed in the rat.

In cottonseed flour it is largely associated with the denatured (salt-insoluble) protein fraction.

Evaporation of an ether solution of gossypol on isolated cottonseed protein does not bind the gossypol to the protein (see reference 4) as in the moist cooking of seed.

The percentages of bound gossypol, 1.9 in the rat feces and 1.74 in the flour residue, after salt extraction, are the highest reported in a biological product.

REFERENCES

1. Withers, W. A., and Carruth, F. E. J. Agric. Research, *12*, 87 (1918).

2. Clark, E. P. J. Biol. Chem., *76*, 229 (1928).

3. Gallup, W. D. J. Biol. Chem., *76*, 43 (1928).

4. Jones, D. B., and Waterman, H. C. J. Biol. Chem., *56*, 501 (1923).

5. Swensen, A. D., Fieger, E. A., and Upp, C. W. Poultry Sci., *21*, 374 (1942).

6. Halverson, J. O., and Smith, F. H. Ind. & Eng. Chem., Anal. Ed., *5*, 319 (1933).