much heat treatment, as measured by the extent of protein denaturation (10), as had commercial cottonseed meals. Similar results were obtained with petroleum ether-extracted cottonseed meats.

Two conditions present in commercial cooking practice had not been controlled, i.e., the moisture content and the stirring. Preliminary attempts to control the moisture content during autoclaving were not successful, but increasing the surface of the meal particles, as by ballmilling, gave products which were readily detoxified by autoclaving (8). Typical results are shown in Table IV. Discontinuance of the research program prevented a more detailed study of this phenomenon. Others (7, 14) have since described the importance of the amount of moisture present in cottonseed flakes for their detoxication by steam heat.

TABLE IV

Effect of Ball Milling (24 Hours) on the Susceptibility of the Toxic Factor in Hexane-Extracted Cottonseed to Destruction by Wet Heat (Autoclaving for 50 Minutes at 109°C.)[a]

Treatment	Number Rats	Average Original Weight	Average Change in Weight	Remarks
		gm.	*gm.*	
None	3	47	−14	Av. survival 7 days
Ball-milled	3	47	+99	24 days

[a] The diets contained 48% cottonseed meal (see Diet D). The autoclaved ball-milled meal was considerably darker than the autoclaved 60-mesh-size meal.

The author is indebted to T. D. Fontaine for assistance in these studies.

Summary

The toxic factor in cottonseed can be nullified by three apparently unrelated mechanisms: oxidation, combination with soluble iron salts, and destruction by steam autoclaving. Oxidation is of minor importance except where the toxic factor has first been extracted with ethyl ether and is present in the diet in solution in oil. Detoxication with soluble iron salts is demonstrable even when the iron is administered in the drinking water. Ball-milled petroleum ether-extracted cottonseed meal can be detoxified by autoclaving under conditions which are not effective for the original meal.

These results were obtained from feeding tests with rats.

REFERENCES

1. Gallup, W. D., Ind. Eng. Chem., *19*, 726 (1927).
2. Gallup, W. D., J. Dairy Sci., *10*, 519 (1927).
3. Gallup, W. D., J. Biol. Chem., *77*, 437 (1928).
4. Gallup, W. D., *ibid.*, *91*, 387 (1931).
5. Gallup, W. D., *ibid.*, *93*, 381 (1931).
6. Gallup, W. D., and Reder, R., J. Agric. Res., *51*, 259 (1935).
7. Lyman, C. M., Holland, B. R., and Hale, F., Ind. Eng. Chem., *36*, 188 (1944).
8. Olcott, H. S., U. S. Patent No. 2,316,014, April 6, 1943.
9. Olcott, H. S., and Fontaine, T. D., J. Nutrition, *22*, 431 (1941).
10. Olcott, H. S., and Fontaine, T. D., Ind. Eng. Chem., *34*, 714 (1942).
11. Olcott, H. S., and Mattill, H. A., Chem. Rev., *29*, 259 (1941).
12. Osborne, T. B., and Mendel, L. B., J. Biol. Chem., *29*, 289 (1917).
13. Robinson, W. L., Ohio Agric. Exp. Sta. Bull. No. 534 (1934).
14. Sewell, W. E., Ala. Agric. Exp. Sta. Bull. 259 (1943).
15. Swensen, A. D., Fieger, E. A., and Upp, C. W., Poultry Sci., *21*, 374 (1942).
16. Withers, W. A., and Brewster, J. F., J. Biol. Chem., *15*, 161 (1913).

Germination and Free Fatty Acid in Individual Cotton Seeds[1]

CARROLL L. HOFFPAUIR, DOROTHY H. PETTY, and JOHN D. GUTHRIE,
Southern Regional Research Laboratory, New Orleans, Louisiana[2]

SEEDS from cotton that has been exposed to wet weather in the field are likely to be lower in viability and to contain higher percentages of free fatty acids than those from seed cotton harvested without unfavorable exposure (3, 4). Similar observations have been made of cottonseed stored under conditions of high moisture or temperature (4). Conventional methods of approach to the relationship of free fatty acid content to germination would require that a sample of several hundred grams of cottonseed for the free fatty acid determination and another sample of several hundred seed for germination tests be drawn from each lot tested. When sufficient data were obtained, statistical methods could be used to study the relationship between the two variables. A second approach to the problem consists of the application of microchemical methods to the analysis for the free fatty acid content (2) of part of the nongerm portion of a single seed and the germination of the remainder of the seed.

In order to establish whether the free fatty acid content of the nongerm end of a hulled cotton seed was correlated with that of the germ end, 50 seeds were carefully peeled and cut approximately in half; each half was weighed and placed in a numbered, small, glass-stoppered Erlenmeyer flask. To each flask 5 ml. of petroleum ether (American Oil Chemists' Society, Specification H 2-41) was added and allowed to stand for about 30 minutes to soften the seeds. The seeds were then ground by means of a glass rod with a flattened end. Any material adhering to the rod was washed into the flask by means of an additional 5 ml. of the petroleum ether. The flasks were then stoppered and allowed to stand for about 16 hours with occasional shaking. After the extraction was completed, 10 ml. of neutralized alcohol containing m-cresol purple indicator was added and the mixture immediately titrated with 0.005 N alcoholic KOH. During the titration the effect of atmospheric carbon dioxide was eliminated by bubbling a stream of carbon dioxide-free air through the titration flask. The free fatty acid content is calculated as per cent oleic acid by multiplying the milliequivalents of alkali used by 28.2 and dividing the product by the weight

[1] Republished with permission from Science, *106*, No. 2754, October 10, 1947.

[2] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

of the portion of kernel extracted. These values may be put in terms of the free fatty acid in the oil by multiplying by 3 since the kernels contain approximately one-third oil.

The fatty acid content of the germ ends varied from 0.4 to 23.6% and averaged 6.9%; that of the nongerm ends, from 0.4 to 23.8% with an average of 7.2%. The correlation coefficient was 0.79, indicating a highly significant, though not by any means perfect, correlation between the free fatty acid contents of the two ends of a cotton seed. Analysis of the composited germ ends and composited nongerm ends of several hundred cottonseed showed that the nongerm end contained 39.5% oil, while the germ end contained 38.7%.

The seeds used to obtain the data plotted in Fig. 1 were selected from 10 sample lots of seed: three lots from the 1941 crop, five from the 1942 crop, and two from the 1943 crop. The seeds used were from experimental plots grown at Tifton, Georgia, Florence, South Carolina, Knoxville, Tennessee, and Baton Rouge, Louisiana, and included the following varieties: Coker's Farm Relief No. 5, Coker's 100 str. No. 3, Acala 1-13-3-1, Rowden 42A, Stoneville 37-10, and Arkansas Green Lint. The seeds were carefully hulled by hand using a razor blade. They were then cut approximately in half perpendicular to the long axis. The germ end was sterilized by dipping in a solution containing 0.25 gram of mercury bichloride dissolved in a liter of 50% ethanol (1). After being rinsed in sterile distilled water, the germ end was almost completely submerged, pointed end down, in sterile nutrient agar in a numbered test tube, and after being covered with a sterile cap the tube was placed in the dark to germinate. When growth above the agar, accompanied by root formation, was noted, germination was rated positive. If such growth was not observed within two weeks, the seed was rated dead.

The nongerm end was weighed, placed in a numbered flask, extracted, and titrated as described above.

In Fig. 1 are shown the results obtained from 369 individual cottonseeds. Several dead seeds were found whose nongerm end contained less than 1% fatty acid. These may have been seeds which were nonviable from some cause which did not produce an increase in acidity or seed in which acidity development was higher in the germ end than represented by the analysis of the nongerm end. All seeds whose nongerm end contained over 5% of fatty acids were dead. A striking feature of the data is that so few seeds were found whose nongerm end contained from 2.0 to 4.0% fatty acid. Only two seeds were found, probably indicating a rapid rise in fatty acid content in this range. Two seeds having fatty acid content in the nongerm end in excess of 4.0 per cent were found to be viable. Since these showed some discolored spots, the value for fatty acid obtained on the nongerm end is probably considerably higher than that of the germ end. The data indicate that most (over 71%) of the seeds contained less than 1% of free fatty acid, whereas the fatty acid content of the others ranged from 1 to 30%. In the group of seeds containing less than 1% the number of live seeds was about 21 times the number of dead seeds, while in that containing from 1 to 30% there were 14 times as many dead as live seeds.

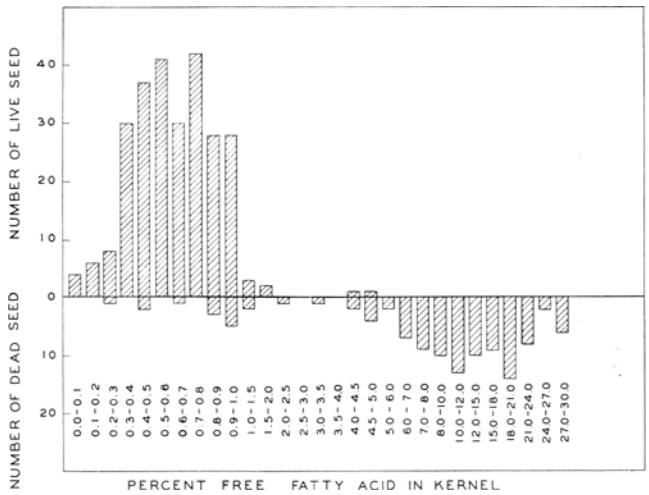

FIG. 1. Distribution of viable cottonseed according to per cent free fatty acid in kernel.

REFERENCES

1. Arndt, C. H., Plant Physiol. *20*, 200, 1945.
2. Hoffpauir, C. L., and Kettering, J. H., Amer. Dyestuff Rep., *35*, 265-266, 1946.
3. Meloy, G. S., Oil & Soap *16*, 174, 1939.
4. Simpson, D. M., *J. Agric. Res.*, *64*, 407, 1942.