point in the ranges covered. Intermediate values were then interpolated, assuming a linear function, with the result that some may vary from the exact color component values by 0.01 A.O.C.S. color unit. Since the uncertainty in each of the four color component terms averages about .06 color units, corresponding to an actual uncertainty of 0.2% in the transmittance readings, it is apparent that the discrepancy of .01 color unit is without practical significance. Moreover when we realize that the standard deviation of A.O.C.S. photometric color readings amounts at this time to around 0.2 to 0.3, it did not seem worth the extra time and effort involved to calculate all the fractional values in the table to the third decimal place just to eliminate a discrepancy of .01 in some of them. It may be mentioned that we deliberately arranged the data so that all four tables come within the compass of standard 8½ x 11 in. office paper so that for protection against hard usage it can be slipped into a celluloid envelope designed for that size sheet. Indeed, by a slight photo-reduction the typed tables all go on one side of that size sheet.

A word might be added concerning our preference for reading the spectrophotometer in terms of percentage transmittance. In the first place, it seems more natural to read a horizontal scale from left to right, as in the case of the slide rule. Another advantage is in the fact that the transmittance scale is of uniform scale division size. Estimation to the nearest tenth is the same on all parts of the scale. Other users have expressed the same preference for reading in terms of transmittance.

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The Storage of Cottonseed. IX. Behavior of Cottonseed During Storage Under Mill Conditions[1]

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IN a series of experiments, carried out with the cooperation of industry during the years 1944 through 1950, an investigation has been made on the behavior of cottonseed of various moisture contents stored at commercial mills. Previous reports of observations on the heating of cottonseed and the chemical changes that take place in this commodity during storage have been made by Malowan (1, 2, 3) on relatively small quantities of seed and by Altschul *et al.* (4, 5, 6, 7) in connection with the chemical treatment of cottonseed to prevent deterioration. No detailed account has been found in the literature of the damage that occurs in piles of cottonseed (up to 40 tons) during storage. It is the purpose of this paper therefore to present the changes that occur as a result of storage: such as heating, the formation of free fatty acids, variations in chemical composition, and the increases in the refining loss and color of the refined oil. The experiments reported here have been made on 20- to 40-ton lots of seed drawn from the daily receipts of the various mills that cooperated in this investigation.

Experimental Procedure at the Mill

The following procedure was adhered to in each of the mills where special bins were erected to house the seed. The bins were built of wood and lined with building paper (15-pound asphalt impregnated felt). Each bin had a built-in false bottom approximately one foot from the floor which permitted aeration of the seed pile on installation of a suitable suction fan. Generally aeration was applied whenever the temperature of the seed exceeded 90° to 100°F. The temperature at various levels within a single bin was measured by thermocouples which were connected to a potentiometer *via* a multiple point handswitch. A record of the daily temperatures registered by each thermocouple inside the bin was forwarded to the Southern Regional Research Laboratory along with a record of the atmospheric temperature.

In a majority of the experiments, samples of seed were withdrawn with a screw auger from several spots in the bin at weekly intervals. The small samples were combined and thoroughly mixed before one-half of the composite was forwarded to the laboratory for analyses for moisture and free fatty acids contents.

Uniformity in the moisture content of the seed was desirable and was attained by selection of seed of one moisture content from the daily receipts. As a result, the experimental lots of seed were not necessarily similar to the regular daily collections of any one mill at any given time.

Two methods of collecting the experimental seed were followed. In one, receipts were set aside in a large pile in an empty storage house until a volume of approximately 60 to 75 tons had been collected. In the other, the stream of seed being unloaded from the trucks was diverted to the storage bin. In most cases this stream of seed was divided in two, and one portion fell into the storage bin while the other was directed to an empty spot in the storage house to be processed as soon as possible after the beginning of the experiment.

In those experiments where a large bulk of seed was collected first, one-half of this seed pile was

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[4] This work was conducted with these companies under an informal memorandum of understanding with the Bureau of Agricultural and Industrial Chemistry.

TABLE I

Location, Volume, Depth, and Initial Free Fatty Acids and Moisture Contents of Seed-Lots Stored in Commercial Mills

Year	State	Volume	Depth	Initial free fatty acids content[a]	Initial moisture content
		tons	*feet*	%	%
1948	Mississippi	0.5	3.5	0.4	12.0
1946	Louisiana	20	8	1.1	14.3
1945	Louisiana	40	17.5	7.0	13.6
1944	Mississippi	30[b]	16	5.0	13.8[c]
1947	Louisiana	20	8	0.7	17.9
1945	Georgia	28	11.5	2.6	18.7

[a] Expressed as percentage of oleic acid.
[b] Capacity of bin.
[c] The moisture content of this seed was raised artificially from 9.0% to 13.8% by the addition of water.

processed immediately and composite samples of the seed, oil, and cake were obtained. Sampling of the seed which was processed immediately always followed the same pattern. Pint lots of seed were withdrawn at 15-minute intervals as the seed was being conveyed from the storehouse. In sampling the oil, one or two presses were selected and all of the oil being expressed on these presses was collected in drums. The oil in each drum was well mixed and allowed to stand for approximately 16 hours. The oil layer was then separated from the settlings and mixed thoroughly. Sampling consisted of removing five gallons from each drum, and if two drums or more had been collected, the oil from each drum was pooled and mixed before a five-gallon lot was withdrawn. From this smaller volume of oil one-gallon portions were poured off. Samples were reserved for the mill and duplicates were sent to the laboratory for analyses. One-half of the cake from each press for every pressing cycle was set aside and combined into a large sample from which approximately 80 to 100 pounds of meal were obtained. Essentially the same procedure was followed at the end of the test during the processing of the stored seed.

Analytical Methods

Determinations for moisture, free fatty acids, oil content, ammonia, refining loss, and refined oil color were made according to the methods of the American Oil Chemists' Society (8).

Estimations of the number of microorganisms per gram of whole seed were made by mixing five gr. of fuzzy cottonseed in 100 cc. of sterile 0.05% aqueous Tween 20[5] in a Waring[5] blendor for three minutes. Serial dilutions of the resultant suspension were plated in nutrient agar (Difco[5]) for the aerobic bacterial count and in a modified Czapek's medium for the mold count.

Estimations of the percentages of internally infected and viable seeds were obtained by acid delinting samples of 50 seeds with concentrated sulfuric acid for two minutes, rinsing in tap water, surface sterilizing with 0.1% silver nitrate solution for three minutes, and planting the seeds on modified Czapek's agar in petri dishes. Observations of all plates were made after five days' incubation at room temperature.

Effect of Storage on Seed

A total of 12 experiments on mill-scale lots of cottonseed have been conducted over a period of six years. According to the data for heating and the formation of free fatty acids in these various lots of seeds, there are five different categories into which these experiments may be divided, depending on the initial moisture and free fatty acids contents of the seed. These categories illustrate: 1) seed having a low moisture content as well as a low free fatty acids content; 2) a medium moisture content and low free fatty acids content; 3) a medium moisture content and a high free fatty acids content; 4) a high moisture content and low free fatty acids content; and 5) a high moisture content and a high free fatty acids content. The experiments selected as illustrations of the five categories are listed in Table I and are identified according to the year in which the test was made as well as the state where the experiment took place. With one exception, each category is represented by a single experiment in which naturally moist seed has been used. The third category is illustrated by two experiments, one in which naturally moist seed was used and the other in which seed was artificially conditioned from a moisture content of 9% to 13.8%.

Heating. Figure 1A shows that cottonseed of low initial moisture and free fatty acids contents did not heat. In seed of higher initial moisture and free fatty

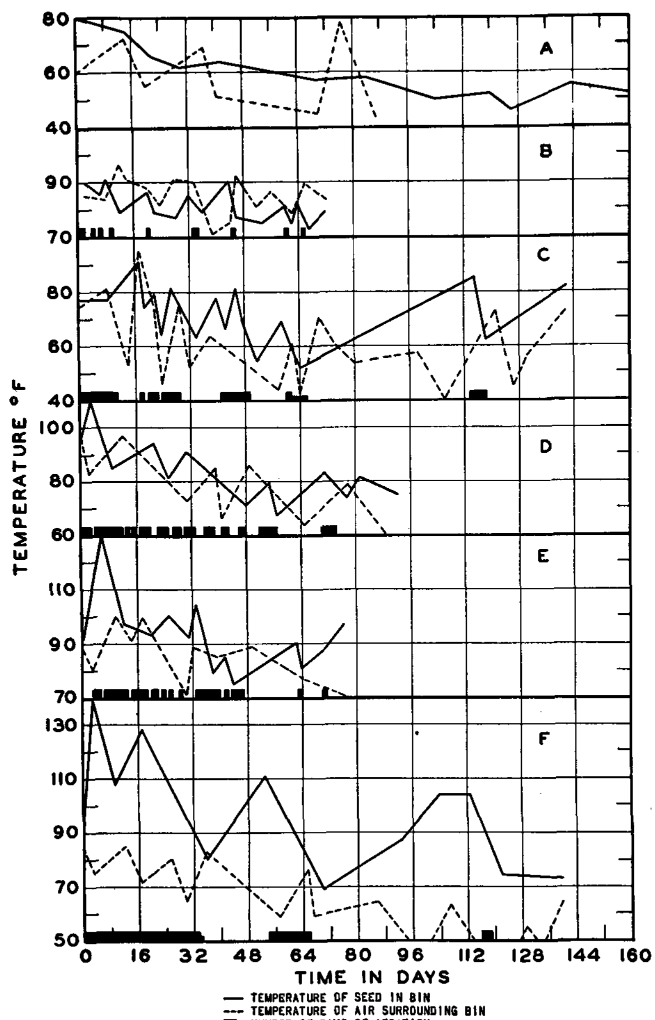

FIG. 1. Heating of 20- to 30-ton lots of cottonseed of various initial moisture and free fatty acids contents.

Curve	Moisture	Free fatty acids	Curve	Moisture	Free fatty acids
	%	%		%	%
A	12.0	0.4	D	13.6	7.0
B	14.3	1.1	E	17.9	0.7
C	13.8	5.0	F	18.7	2.6

[5] The use of trade names in this article is for identification only and implies no endorsement of the manufacturer or the product.

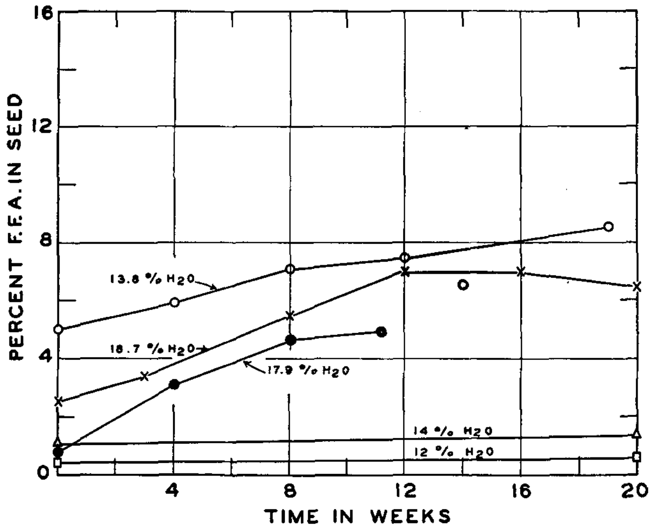

FIG. 2. Production of free fatty acids during storage of mill-scale lots of cottonseed of varying moisture contents.

acids contents there was an increasing tendency to heat and aeration was required to control the temperature. (See cross hatching at the bottom of the individual graphs in Figure 1.) It will be noted that increases occurred not only in the total number of days of aeration but also in the number of days of continuous aeration necessary to reduce and maintain the temperature at 80°F. Of the two lots of seed belonging to the third category, naturally moist seed (Figure 1D) displayed a greater tendency to heat than artificially moistened seed (Figure 1C).

Free Fatty Acids Formation. The seed lots that heated most and required the most cooling were also the ones which developed free fatty acids fastest (Figure 2). Seed of initially low or medium moisture and low free fatty acids contents developed little or no free fatty acids. Seed of high initial moisture content, regardless of the initial free fatty acids content, produced free fatty acids at a relatively high rate. Naturally moist seed of medium initial moisture (13.6%) and high initial free fatty acids (7.0%) contents produced free fatty acids at a rate comparable to that of high moisture seed in as much as the quantity of free fatty acids increased from 7.0%-12.0% in three months of storage. Free fatty acids were produced at approximately half that rate in the artificially moistened seed (13.8% H_2O curve, Figure 2).

Effect of Aeration. In addition to cooling the seed, aeration reduced the moisture content until the seed was at equilibrium with the surrounding atmosphere. When this was accomplished, the rate of formation of free fatty acids decreased significantly. To demonstrate this secondary effect of aeration (Figure 3), an experiment conducted in Louisiana in the 1949 season was selected. The seed stored for this test had an initial moisture content of 17.9% and an initial free fatty acids content on arrival at the mill of 2.9%. When analyzed at the Southern Regional Research Laboratory a week later, the free fatty acids content of the seed was 3.7%. Since similar delays occurred for all of the subsequent samples, the value 3.7% was used as the initial free fatty acids content in Figure 3.

It will be noted that 23 days of continuous aeration were required to control the heating of the seed during the first month of storage (Figure 3). During this period a 2.5% increase in the content of free fatty acids occurred. Control of the temperature therefore did not stop the production of free fatty acids.

Microbial Population. In seed of medium moisture (13.3%) but high free fatty acids content (3.3%), initial bacterial and mold contaminations were rather high (Table II). However it appears that no significant increase in population occurred as a result of aeration nor did any appreciable decrease take place during storage for 69 days. The moisture content of this lot of seed was comparatively low and even though the temperature of the seed entering the bin was 95°F., only three days of aeration were necessary to control the temperature. It is believed that the low moisture content of the seed which corresponds to a relative humidity of just under 65% (9) in the inter-seed air is responsible for the fact that no appreciable changes took place in the microbial population during storage (10). Estimations of viability made on seed sprouting on acid agar, show no loss in germinability during storage.

Ammonia and Oil Contents. Samples of seed were analyzed for the percentages of oil, ammonia, moisture, and free fatty acids before and after storage. The ammonia and oil contents are customarily reported on a fuzzy seed "as is" basis for the purpose of calculating the quantity index. Where it is necessary to determine if changes occurred in these components as a result of storage, comparisons should be made on a dry weight basis of the kernel. When that is not possible, the dry weight of the whole seed should be used. A comparison of the percentages of ammonia and oil on a wet and dry basis of the whole seed and of oil in the kernels is made in Table III.

If the initial ammonia contents of the whole seed are reported on a wet basis, there appears to be an increase in that component during storage of the seed. If these values, listed in column 7, Table III, are recalculated on a dry weight basis of the whole seed, column 8, it appears that no significant change occurs in the ammonia content.

It will be noted in column 9, Table III, that if the oil content is reported on the fuzzy seed "as is" basis, there is apparently little or no loss of oil, and

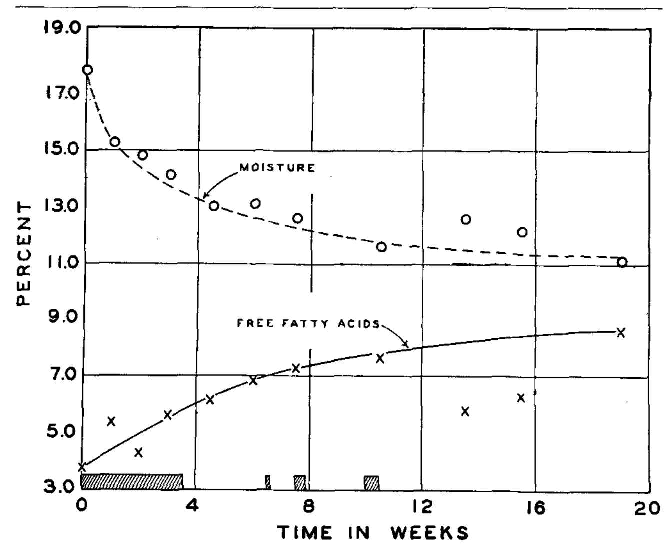

FIG. 3. Changes in moisture content and free fatty acids content during storage of cottonseed in Louisiana in 1949.

TABLE II

The Effect of Storage on the Microbial Population of 25 Tons of Cottonseed Used in an Experiment in Georgia During the 1948 Season

Length of storage	Description	Moisture content	Free fatty acids content [a]	Oil content [b]	Infection [c]	Bacterial count [d]	Mold count [d]	Germination [e]
days		%	%	%	%	*millions*	*thousands*	%
0	Initial processing control	13.3	3.3	18.1	100	17.0	1000.0	54
0	Bin sample	13.0	3.4	18.6	100	7.0	600.0	54
4	Bin sample after aeration	13.2	....	....	100	9.0	500.0	44
69	Bin sample after storage	11.1	6.0	18.7	72	2.0	2600.0	48

[a] Expressed as percentage of oleic acid. [b] Oil content on fuzzy seed "as is" basis. [c] Percentage of seeds infected internally. [d] Numbers of microorganisms per gram of whole seeds on an "as is" basis. [e] Germination of acid-delinted surface sterilized seed on Czapek's medium containing 1% dextrose in place of sucrose, pH = 5.

in some instances a gain of oil during storage. If the oil content of the fuzzy seed is recalculated to a dry weight basis of the seed as in column 11, an entirely different trend is noted. In most cases there is actually a loss of oil during storage. In more recent tests it has been our practice to determine the moisture content of meats at the time when oil extraction begins. If the figures in column 12 for the 6th, 7th, and 9th tests are examined, it will be noted that during these tests losses in oil content have been observed to the extent of 0.8% to 4.0% in the meats on a dry weight basis. Further there is an indication that the loss in oil increases as the interval of storage increases.

Effect of Storage on the Products

The products to be considered here are the oil and the cake. Observations on the effect of storage on the quantities of these components have already been made in the section concerned with the effect of storage on the seed.

Oil. It was noted that the increases in refining loss bore no relationship to the length of time of storage. Rather the single factor which influenced these increases was the quantity of free fatty acids developed in the oil during storage. The free fatty acids and refining losses of the oil from fresh and stored seed, enumerated in Table IV, illustrate the well-established relationship between free fatty acids content and refining loss (11, 12).

It was observed (Table IV) that when there was an increase in the free fatty acids content of the crude oil, there was also an increase in the Lovibond red color of the refined oil. However no relationship between the increase in red color and the interval of storage was found.

Meal. In Table III, it was shown that only negligible differences in ammonia content occurred. Therefore no loss took place in the protein content of the meal as a result of storage.

Discussion

Both prime and off quality seeds were selected for this series of experiments. Weekly determinations for the percentages of moisture and free fatty acids indicated changes in quality of the seed during storage. Daily temperature measurements made it possible to control the production of heat during each experiment. This control was maintained by the application of sustained aeration whenever it was required. These periods of sustained aeration not only cooled the seed but also reduced the moisture content appreciably. The net effect was to reduce the rate of deterioration. While these experiments have been

TABLE III

Changes in Oil and Ammonia Contents During Storage

Test		Storage	Moisture content		Rate of formation of free fatty acids	Ammonia content [b] of fuzzy seed		Oil content on "as is" basis		Oil content on dry wt. basis	
Year	State		Fuzzy seed	Kernel		"As is"	Dry wt.	Fuzzy seed	Kernel	Fuzzy seed	Kernel
		days	%	%	%/mo.	%	%	%	%	%	%
1944	Louisiana	0	15.8	......	1.31	3.76	4.46	17.8	......	21.1	38.0[a]
		114	10.5	......		4.40	4.91	20.5	......	22.9	41.2[a]
1944	Mississippi	0	13.8	......	0.71	3.87	4.48	18.9	......	21.9	39.4[a]
		143	11.7	......		4.01	4.54	18.9	......	21.4	38.5[a]
1945	Georgia	0	18.7	......	0.87	3.53	4.34	16.8	......	20.7	37.3[a]
		145	12.2	......		3.91	4.45	18.2	......	20.7	37.3[a]
1945	Louisiana	0	13.6	......	1.61	3.58	4.14	19.0	......	22.0	39.6[a]
		93	10.6	......		3.80	4.25	19.6	......	21.9	39.4[a]
1946	Georgia	0	15.2	......	0.36	3.67	4.32	18.5	......	21.8	39.2[a]
		177	11.0	......		3.84	4.31	18.6	......	20.9	37.6[a]
1947	Georgia	0	18.6	5.4	0.74	3.47	4.26	16.6	35.6	20.4	37.6
		70	11.6	7.9		3.81	4.30	18.0	33.9	20.4	36.8
1947	Louisiana	0	17.9	5.8	1.55	......	......	16.8[a]	34.7	20.2[a]	36.8
		80	12.7	9.4		......	......	16.9[a]	32.0	19.4[a]	35.3
1948	Georgia	0	13.0	......	1.13	3.66	4.20	18.6	29.9	21.4	38.5[a]
		69	11.1	......		3.82	4.29	18.7	30.3	21.0	37.8[a]
1949	Louisiana	0	17.9	13.8	1.11	3.29	4.00	18.2	33.2	22.2	38.4
		132	11.1	8.1		3.74	4.20	19.7	31.9	22.2	34.4

[a] Calculated value. [b] Nitrogen calculated as ammonia.

TABLE IV
Effect of Storage of the Seed on the Refining Losses of the Crude Oils and Colors of the Refined Oils

Year	State	Storage	Oil analyses		
			Free fatty acids [a]	Refining loss	Refined oil color
		days	%	%	*Lovibond red units*
1947	Louisiana	0 80	0.9 4.7	5.6 11.6	5.2 8.8
1946	Louisiana	0 77	1.2 2.1	5.1 5.9	5.5 6.1
1947	Georgia	0 70	2.2 6.8	9.9 17.4	8.7 8.6
1946	Georgia	0 177	2.4 4.9	10.1 14.7	8.1 11.0
1945	Georgia	0 145	3.0 8.2	11.1 21.6	8.8 21.3
1949	Louisiana	0 132	3.8 8.8	10.2 21.2	13.0 14.9
1948	Georgia	0 69	4.5 6.7	13.7 17.1	10.2 12.0
1945	Louisiana	0 93	9.0 13.1	22.0 32.9	14.7 32.0

[a] Expressed as percentage of oleic acid.

conducted under conditions which approach those in a commercial mill, some control measures, such as aeration for prolonged periods, were actually far better than can be realized in everyday mill practice. Therefore it might be expected that seed of the quality stored during these tests would deteriorate more rapidly under current methods of handling.

Cottonseed of moisture contents in excess of 12% have been shown to heat. Also, the tendency to heat, i.e., the rapidity with which the seed heats and the temperature that is attained, increases as the initial moisture and free fatty acids contents increase. The rates at which free fatty acids are formed in cottonseed during storage are similarly influenced by the initial moisture and free fatty acids contents of the seed. Therefore the intensity of biological activity as measured by the production of heat and the rate of formation of free fatty acids was found to be dependent upon the initial moisture and free fatty acids contents of the seed.

One experiment in this group involved a lot of artificially moistened seed of approximately the same initial moisture and free fatty acids contents as those of a naturally moist lot of seed. The temperature attained by the naturally moist seed was considerably higher than that reached by the artificially conditioned seed. Similarly, the rate of formation of free fatty acids in the naturally moist seed was twice that of the artificially conditioned seed. These facts are interpreted to mean that moisture added artificially does not recreate conditions in the seed entirely comparable with those that exist if the moisture is of natural origin.

As mentioned previously, the intervals of sustained aeration, although not common practice in the mills, cooled the seed and reduced the moisture content appreciably. In a number of cases the storage period was long enough and the aeration applied efficient enough to reduce the moisture content of the seed to a level where it was in equilibrium with the surrounding atmosphere. When this was accomplished, the rate of formation of free fatty acids decreased significantly. Simultaneously, the tendency to heat was observed to diminish; sustained aeration for lengthy periods was no longer required; and, in some instances, aeration was dispensed with entirely. It would appear then that the intensity of heating may be controlled to some extent by lowering the moisture content of the seed to that level optimum for processing. However, at present, it is not possible to remove moisture rapidly enough from a seed pile during the early stages of storage to control the initial rapid rise in the content of free fatty acids in the seed.

Recently Whitten (13) reported a loss in oil content in cottonseed during prolonged storage of 270 days and associated this loss with an increase in moisture content. In the tests discussed above, reductions in moisture content have been accomplished by aeration. Nevertheless there has been a loss in oil during storage. Therefore it appears that neither an increase nor a decrease in the moisture content contributes to the loss in oil. It is indicated from the data at hand that loss in oil is proportional to the interval of storage. This statement is based on laboratory data only but seems of sufficient interest to warrant further investigation by storing a large volume of seed for a prolonged period during which time sufficient tonnage could be removed at definite intervals to check the actual loss in oil under mill conditions.

Summary

The production of heat and the rate of formation of free fatty acids in 20- to 40-ton lots of cottonseed have been found to be dependent on the initial moisture and free fatty acids contents of the seed. Even though sustained aeration controlled the temperature of the seed and reduced the moisture content appreciably, the rate of formation of free fatty acids was rapid in all the tests except those involving seed of initially low or medium moisture and low free fatty acids contents. Losses in oil content proportional to the storage interval occurred.

Acknowledgment

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