The Absence of Lipase in Cottonseed[1]

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COTTONSEEDS contain approximately 20% lipids. Under normal conditions samples can be stored for long periods of time without deterioration of the oil. However, if the seed contains excess moisture, the free fatty acid content frequently increases during the storage period, in extreme cases to as much as 40% of the total oil content. This development of acidity is accelerated by the spontaneous heating of piles of wet cottonseed, a phenomenon similar to that observed with wet wheat or corn and attributed to increased respiration stimulated by the excess moisture (9). In recent years, losses in oil values due to cottonseed deterioration have been reduced by improved seed house designs permitting aeration of wet seed.

Malowan (4) stated that seed with less than 10% moisture rarely heats and seed with more than 12% moisture has to be cooled to prevent heating. Robertson and Campbell (7) concluded that seed of 10-14% moisture content may or may not remain stable while samples having more than 14% moisture deteriorate in storage with rapid increase of free fatty acid. Freyer (2) correlated moisture content with free fatty acid development and found that above 12.5% water content the chances of deterioration are greatly increased. He pointed out, however, that the presence of excess moisture in a particular sample of seed does not indicate that free fatty acid development will occur.

Meloy (5) concluded from a study of numerous analytical data that the enzymes of cottonseed are rendered inactive by the dehydration which normally occurs shortly after the opening of the boll. When this process is delayed as in an excessively wet season, enzymic activity continues and free fatty acids develop. If normal dehydration does occur, the enzymes are not so readily re-activated by subsequent increases in moisture content. This theory might account for the variability in behavior of samples of high moisture content seed (Table 1).

TABLE I.

Development of Free Fatty Acid in Wet Cottonseed[1]

Date Harvested	Date Ginned	Moisture and F.F.A. Content: Original H_2O%	Original F.F.A.%	After 90 Days Storage H_2O%	After 90 Days Storage F.F.A.%
8-14-39	8-17-39	14.15	0.7	13.90	4.7
8-14-39	8-19-39	15.78	0.7	15.58	29.3
8-15-39	8-21-39	18.78	1.9	14.15	30.8
8-24-39	8-26-39	16.78	0.9	17.42	14.5
9-16-39	9-23-39	17.65	0.9	16.22	17.4
9-22-39	9-24-39	15.25	0.6	12.38	1.4
10- 5-39	10- 5-39	14.23	0.4	11.50	0.5
9- 5-40	9-10-40	17.80	0.8	16.15	9.5
10- 1-40	10- 5-40	15.60	0.3	15.70	0.7
10- 7-40	10- 8-40	18.28	0.3	14.32	15.9
10-15-40	10-19-40	15.75	0.3	14.98	0.4
11- 4-40	11- 6-40	14.50	0.2	14.05	0.4

[1] These data were kindly furnished by the Delta Experiment Station and U.S.D.A., Stoneville, Miss.

On the assumption that the release of free fatty acid is mediated through the agency of a lipase, investigations to determine the nature and properties of the enzyme were initiated. It was found, however, that, *with the methods used,* no lipase could be detected in cottonseed from *whatever* source or in *whatever* condition unless germination intervened. Inasmuch as cottonseed requires more than 50% moisture for germination (6), it is doubtful whether this enzyme plays any part in the fatty acid development. The properties of the lipase of *germinated* cottonseed have been described elsewhere (6). It is hoped that by recording the following negative experiments other investigators will be saved the necessity of duplicating similar attempts to reveal the mechanism of free fatty acid formation.

Experimental

The method finally adopted for assaying lipase activity was a modification of that described by Longenecker and Haley (3)(6). The preparation to be assayed was weighed into a homeopathic vial. Buffer solution (4 cc.), refined cottonseed oil (1 cc.), and one drop of toluene were added, the vial was stoppered with a paraffined cork and shaken for 16 hours at room temperature. The contents were then titrated in isopropanol-petroleum ether (2:1) solution with sodium hydroxide in isopropanol and aniline blue indicator (1). When large amounts of meal interfered with the sharpness of the end point, the solids were removed by centrifugation and washed with the isopropanol-petroleum ether solvent. Combined centrifugate and washings were then titrated. Appropriate blanks were included in each run. Series of assays were run within the pH range 3 to 11, with different buffers, and with the addition of lipase activators such as calcium chloride. These methods and additions gave the expected positive results with known lipase sources, such as pancreatin, castor bean meal and *germinated* cottonseed preparations, when 10 mgs. or less were used. However, 200 mg. samples of the various cottonseed meals effected no lipolysis.

In Table II are listed some of the preparations which were examined. Wet seed were dehulled and assayed as a suspension of the ground kernel or after drying (desiccator) and extraction. Although the incubated wet seed (fertile seed to which the calculated amount of water had been added) gave consistently negative results, they also did not develop free fatty acid rapidly; hence the certainty was lacking that conditions were similar to those in deteriorating seed. Samples of seed were then obtained which had contained 15.5% moisture from the time they were harvested, and had developed 15.9% fatty acid during the 90-day storage period. These also showed no lipolytic activity, nor did those which had been stored for a year and contained 30% free fatty acid.

Finally, samples of seed which were wet (15.5% moisture) when ginned but had not deteriorated after 90 days' storage were placed in a desiccator containing water, according to the suggestion of Robertson and Campbell (7). As shown in Table III, the rapid development of free fatty acid began about the 16th day. On the 37th day, when lipolysis was actively in prog-

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ress, samples of the seed were assayed for lipase. Ground kernels, ground dried kernels, and solvent-extracted preparations all were used. The assays were completely negative. Ordinary fertile seed (1939 crop) did not deteriorate under the same treatment.

TABLE II.

Cottonseed Preparations Giving Negative Lipase Assays
Ground meats
Ether-extracted meats
Petroleum ether-extracted meats
Commercial meal
Seed incubated with 15-45% H_2O for 1 week at 25°.
Seed incubated with 18-25% H_2O for 2 months at 38°.
High moisture seed, 3 months old, 0.3% free fatty acid.[1]
High moisture seed, 3 months old, 13.6% free fatty acid.[2]
High moisture seed, 1 year old, 30% free fatty acid.[3]
Seed from immature and freshly opened bolls.

[1] Delfos 3506. Harvested 10-17-40, ginned 10-19-40, 15.5% H_2O, 0.3% f.f.a. This and the following samples were furnished through the courtesy of the U.S.D.A. and the Delta Experiment Station, Stoneville, Miss.

[2] Delfos 4729. Harvested 10-7-40, 18.3% H_2O, 0.3% f.f.a. Received 1-25-41, 14.8% H_2O.

[3] Delfos 531-B. Ginned 8-19-39, 16.0% H_2O, 0.7% f.f.a. Stored in cans until 11-18-39, 15.0% H_2O, 23.0% f.f.a. Stored in bags and shipped 8-3-40, 10.0% H_2O, 31.5% f.f.a.

Coker-Clevewilt. Practically the same. On arrival contained 10.4% H_2O, 30.3% f.f.a.

It may be concluded that, if a lipase is responsible for the free fatty acid development, it is present in amounts too small to be detected with the method used. By rough calculation the concentration of lipase in cottonseed must be less than one-six-hundredth of that present in other known lipase-containing preparations. Attempts to repeat the experiments of Theis, Long, and Brown (8), who assayed the lipase of flaxseed by keeping oil, meal, and water is contact for several weeks, were complicated by the putrefaction which invariably resulted when cottonseed meal was used.

All of the cottonseed preparations described possessed the property of splitting small but detectable amounts of triacetin, tributyrin, and benzyl butyrate. It is believed, however, that this esterase is not of importance in free fatty acid development inasmuch as cottonseed oil does not contain the lower molecular weight fatty acids.

The observation of Robertson and Campbell (7) that cottonseed preheated to 80°C. for 8 hours to kill the germ is even more sensitive than live seed to the formation of free fatty acid in a moist atmosphere also suggests that enzymic activity as usually understood does not participate in the reaction, although it is possible that some enzymes might survive such treatment.

TABLE III

Free Fatty Acid Development in Cottonseed Stored in a Water-Saturated Atmosphere at 25°

Days	Wet Seed[1] Moisture %	Wet Seed[1] F.F.A. %	Ordinary Seed Moisture %	Ordinary Seed F.F.A. %
4	16.6	.....	12.3	.....
9	18.1	.....	16.9	.....
12	.....	1.2	.....	0.34
16	.....	3.4	.....	0.40
29	20.5	8.7	18.2	0.40
35	22.1	11.1	.....	0.30
43	23.4	17.8	.....	.....
46	25.4	20.0	21.5	0.36

[1] See Table II, footnote 1. Placed in humid atmosphere, 1-2-41.

As previously mentioned, Meloy (5) suggested that the enzymes of the newly opened boll are inactivated by dehydration. Immature and freshly opened cotton bolls were obtained by air mail from Mississippi (1939) and from Texas (1940). The seeds separated from them contained 20 to 50% moisture but no lipase could be detected either immediately or after storage.

The possibility that the free fatty acids are products of oxidation rather than hydrolysis was eliminated by characterizing a fraction obtained from cottonseed which had undergone deterioration (Table II, footnote 3). The oil, obtained by petroleum ether extraction, was dissolved in ethyl ether and carefully extracted with 0.75% potassium hydroxide solution. The fatty acids were recovered by acidification and extraction with ether. The data in Table IV indicate that the free fatty acids resemble very closely those obtained by alkaline hydrolysis of the entire oil, and justify the conclusion that hydrolysis is at least the main and probably the only source of free fatty acid. The same conclusion holds for the development of fatty acid in rolled meats which had remained exposed to light and air for several months. The data suggest also that the lipolytic mechanism is non-selective for individual fatty acids.

TABLE IV

Comparison of Properties of Free Fatty Acids and Total Fatty Acids in Cottonseed Oils

Source of Oil	Recovery[1] %	Mean Molecular Weight	Iodine No.[2]
From high moisture, high f.f.a. seed[3]			
Free fatty acids	59.5	271	98.8
Total fatty acids	.....	266	98.0
From stale rolled meats (19.5% f.f.a.)			
Free fatty acids	55.5	273	98.4
Total fatty acids	.....	271	99.8

[1] As percentage of total free fatty acids determined by titration of the oil.

[2] Rosenmund-Kuhnhenn pyridine-sulfate dibromide method.

[3] See Table II, footnote 3.

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Summary

Although analyses of free fatty acid fractions obtained from deteriorating cottonseed indicate that they are released by hydrolysis of the glycerides, no lipase could be detected in meals obtained from such seed. Prime cottonseed also was without lipolytic activity (Longenecker and Haley method). The mechanism of the reaction whereby free fatty acids develop in wet seed during storage thus remains obscure.

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