EFFECT OF INHIBITORS ON THE RESPIRATION AND STORAGE OF COTTONSEED

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(WITH THREE FIGURES)

Introduction

One of the earliest attempts to apply chemical treatment as a means of inhibiting the heating and lipolysis of cottonseed during storage was made by BARROW (**4**), who used sodium chloride as the effective chemical agent. The application of 5 per cent. of sodium chloride to moist cottonseed had a twofold effect. First, as a result of the high salt concentration outside the seeds, considerable moisture was withdrawn from within. Secondly, small quantities of the salt which diffused into the seed inhibited the biological processes responsible for heating and lipolysis. MALOWAN (**17**) found that sodium chloride likewise decreased the rate of evolution of carbon dioxide from moist cottonseed and that alcohol, acetic acid, sulphuric acid, and formalin inhibited respiration. He found that certain disinfectants, such as copper sulphate and mercuric chloride, had no effect on the rate of respiration and concluded, therefore, that the carbon dioxide evolution observed under his experimental conditions was caused not by the action of micro-organisms but rather by the action of enzyme systems operating in the seed.

Two of the present authors have reported previously (**13**) that ammonia and hydrochloric acid vapors inhibit lipolysis of the oil in cottonseed. Subsequent experiments indicated that ammonia treatment also effects the spectrum of extracted cottonseed oil and the heating of seed stored in bulk (**1**). This treatment of cottonseed with ammonia vapors, although potentially adaptable to commercial practice, was shown in mill-scale tests to be unfeasible as proposed (**2**).

In the present investigation the effect of ammonia upon a number of properties of both mature and immature cottonseed was measured in order to form a basis for a better understanding of the mechanism of inhibitor action. Since it was also of interest to determine whether the surface sterilization of cottonseed would seriously inhibit respiration and lipolysis or affect the color of the seed oil, a number of chemical agents known to act as inhibitors of microbial growth were also tested.

Materials and methods

The inhibitors used were ammonia, butyl maleimide, 2′-methyl-l-maleanil, Nacconol NR, Emulsol 607, and Emulsol 607M.

Both butyl maleimide and 2′-methyl-l-maleanil are compounds developed for use as disinfectants (**11**). The former, a liquid at room temperature, is able to disinfect pointed steel strips inoculated with *Staphylococcus aureus* when the inoculated strips are suspended above the compound in a closed space for a period of 2 days. Spores of an Aspergillus species, when placed on glass slides and exposed to vapors of butyl maleimide in a closed space for 6 days at 50° C. or for 7 days at 25° C., do not germinate. Somewhat related in structure and properties to butyl maleimide, but less effective as a fumigant, is 2′-methyl-l-maleanil, which is a solid at room temperature.

Nacconol NR is the proprietary name for an alkyl aryl sodium sulfonate preparation which contains 36 per cent. organic matter, and possesses wetting and dispersing properties (**7**). Methods for preparing compounds of this type and descriptions of their chemical composition and structure are given in the patent literature (**8, 9, 10**). In addition to its detergent properties, Nacconal NR has germicidal properties, inasmuch as 1:500 dilution of the compound will destroy cultures of *Staphylococcus aureus* in 72 hours at 37° C. Nacconol NR is effective also in controlling fungus growth.

Emulsol 607, another type of detergent having germicidal properties (**6**), is described as the lauric acid ester of colamino formylmethyl pyridinium chloride. It will destroy cultures of *Staphylococcus aureus* in 10 minutes in a dilution of 1:20,000, and cultures of *Eberthella typhosa* in the same length of time in a dilution of 1:15,000. An indication of its fungicidal activity is given by the fact that it kills the spores of *Trichophyton interdigitale* and of *T. rosaceum* in a dilution of 1:20,000.

Emulsol 607M differs from Emulsol 607 in that it is derived from myristic acid and is a more effective bactericidal and fungicidal agent than Emulsol 607. Emulsol 607M was available and was used in the anhydrous form, whereas the Emulsol 607 was used in the form of a 10 per cent. aqueous solution.

Three varieties of cottonseed were used. Two of these, the "Delfos" and "Coker's 200-strain-1" were the same as used in experiments reported in a companion paper (**14**). The "Delfos" variety was grown in Stoneville, Mississippi in 1942, was fully matured when harvested, and was designated as the 100 series in the paper mentioned. The sample of seeds of the "Coker's 200-strain-1" variety was from bolls which had been harvested before they had properly matured and was previously designated as lot 204. This sample was grown in 1942, in Clemson, South Carolina. The third variety was "Cleve Wilt" grown in Clemson, during the 1943 season. It was fully matured when harvested and is designated as lot 405. Before being used in the inhibition experiments, the seeds were conditioned to a moisture content between 14 and 15 per cent. in the manner described in other papers (**13, 14**).

Ammonia was introduced by diffusing the gas through the seeds in a closed container for 1 hour. The seeds were then kept in the container (24 hours) to permit them to come to equilibrium with the excess of ammonia that had been adsorbed by the fuzz on the seed. At the end of this period, the pH of the seeds was approximately 8. In some of the experiments reported, similar supplementary treatments with ammonia were given the seed during the course of the storage period.

Butyl maleimide was introduced by exposing 5 pounds of cottonseed for 1 week to the vapors emanating from 20 ml. of this substance. Exposure was accomplished by placing the seeds and the butyl maleimide in a desiccator which was then evacuated and sealed. At the end of the exposure period, the seeds were removed and placed in an air-tight container.

Inasmuch as Nacconol NR, 2′-methyl-l-maleanil, and Emulsol 607M were solids, treatment with these substances was accomplished by thoroughly mixing the seeds with the powdered material in the following proportions: One hundred grams of Nacconal NR with 5 pounds of seed, 20 gm. of 2′-methyl-l-maleanil with 5 pounds of seed; and 10 gm. of Emulsol 607M with 3 pounds of seed. Treatment with Emulsol 607 was accomplished by spraying 5 pounds of the seeds with 25 ml. of the solution in the form of a fine mist.

The treated seeds were stored in air-tight bottles at room temperature. Samples were removed periodically and examined. The analytical methods used for the determination of respiration intensity, lipolysis rate, pH, and spectrum of the seed oil were the same as described in other papers (**1, 3, 13, 14**).

Effect of inhibitor on respiration

In a series of respiration experiments conducted on the "Delfos" variety (100 series), all the chemical agents mentioned above, except Emulsol 607M, were used.

In its effect on the pattern of respiration, ammonia differed from all of the other chemical agents tested. This is evident from the representative respiration patterns of seeds of approximately the same moisture content which are shown in figure 1. The ammonia-treated seeds exhibited a relatively high initial respiration intensity followed in the intermediate portion of the storage period by low respiration, which began to rise again after 200 days' storage. The seeds which were treated with Nacconol NR, however, exhibited a maximum in respiration intensity during the storage period. Similar respiration patterns were given by the seeds treated with Emulsol 607, butyl maleimide, and 2′-methyl-l-maleanil. Such behavior is typical of most of the respiration experiments reported in a companion paper (**14**) (cf. figs. 1, 2, and 3).

The relationship between the ammonia concentration (pH) and the respiration intensity is apparent from an examination of table I, in which are given pH values for the ammonia-treated samples during the storage period. Sample 1 (average pH, 8.03) reached a pH of 8 at the end of the initial treatment and was maintained at or above that level for 3 months. This was in part due to the fact that additional ammonia treatments were given during the storage interval, as indicated in the table. After 150 days' storage, the pH of the seeds was slightly below 8, and from then on it dropped steadily to a final value of 7.75. Sample 2 (average pH, 7.91)

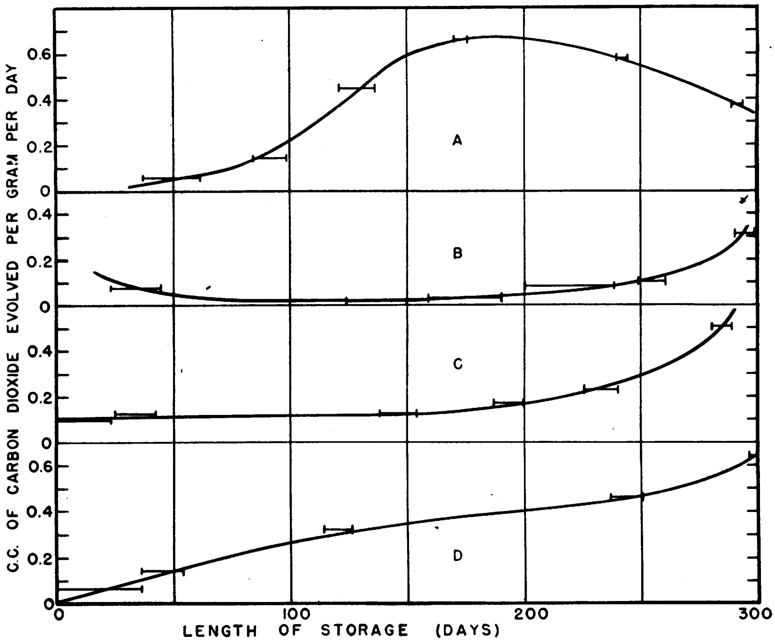

FIG. 1. Effect of inhibitors on the respiration pattern of "Delfos" variety cottonseed (100 series).

Curve	*Inhibitor*	*Moisture content, per cent.*
A	Nacconol NR	14.38
B	Ammonia (average pH of 8.03)	14.86
C	Ammonia (average pH of 7.91)	14.76
D	None	14.60

attained a slightly lower pH after the initial treatment than did sample 1, was maintained briefly at a pH above 8, and then was allowed to drop again to lower pH values. The initial stimulation of respiration indicated in

TABLE I

VARIATION OF pH IN STORED AMMONIA-TREATED COTTONSEED

LENGTH OF STORAGE	SAMPLE 1	SAMPLE 2
days	*pH*	*pH*
0	8.00	....
4	8.05	7.80
25	8.10*	7.85*
32	8.00	7.77
53	8.23*	8.09*
71	8.23*	8.16*
90	8.17	8.12
147	7.94	7.83
218	7.88	7.77
326	7.75	7.79
Average	8.03	7.91

* These determinations were made following supplementary ammonia treatments.

curve B, figure 1, coincides with the initial heating observed following ammonia treatment (**1**). Such an initial stimulation of heating also occurred in mill-scale experiments involving the use of ammonia to improve the storage properties of cottonseed (**2**).

The respiratory patterns of the seeds treated with the inhibitors were integrated in the manner described (**14**) and their average respiratory intensities (RI) were determined. These values are compared in figure 2

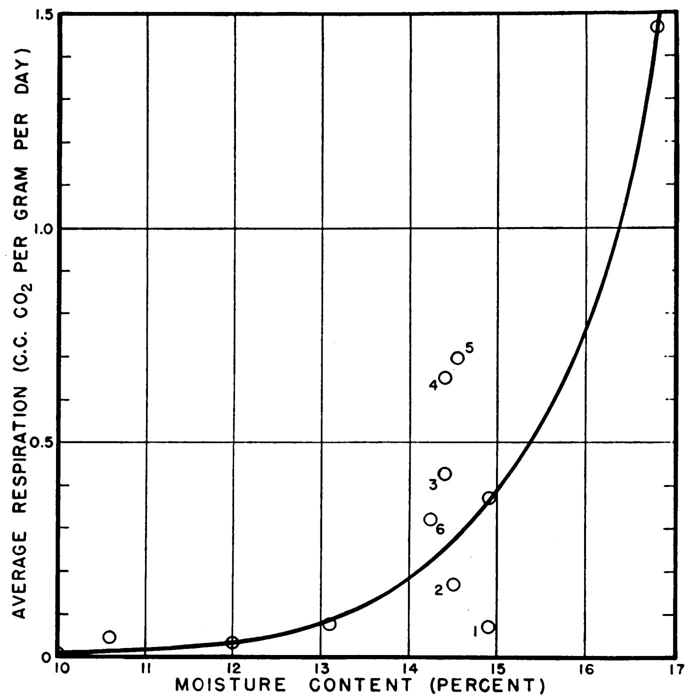

FIG. 2. Effect of inhibitors on the respiratory intensity of "Delfos" variety cottonseed (100 series), the curve representing the respiration of cottonseed in the absence of inhibitors.

1. Ammonia (average pH of 8.03)
2. Ammonia (average pH of 7.91)
3. Nacconol NR
4. Butyl maleimide
5. Emulsol 607
6. 2′ methyl-1-maleanil

with those attained on untreated seeds. The curve for untreated seeds shows the effect of moisture content on the respiratory intensity of "Delfos" variety seeds (100 series) as given in figure 4 of (**14**). It is interesting to note that only treatment with ammonia produced a lower-than-normal average respiratory intensity. The other treatments resulted in an overall stimulation of respiration, regardless of the form in which the fungicide or bactericide was applied.

Effect of inhibitors on lipolysis rate

Free fatty acid determinations were made on the same lots of seeds used for the respiration measurements, and the lipolysis rate constants (**13**) were calculated. These are compared in figure 3 with those obtained on normal untreated seeds of the same series. The curve is that for the "Delfos" variety cottonseed (100 series) which was given in figure 1 of a companion paper (**15**).

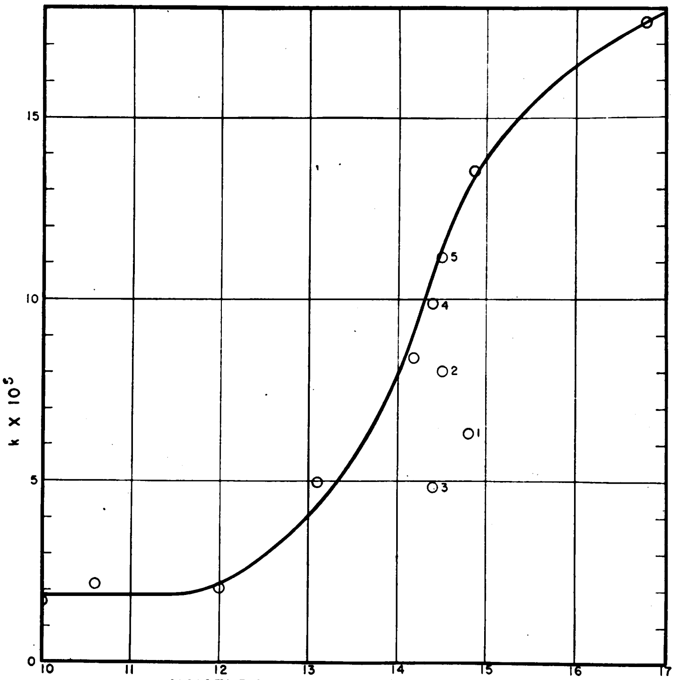

FIG. 3. Effect of inhibitors on lipolysis rate constant, k, of "Delfos" variety cottonseed (100 series), the curve representing lipolysis of cottonseed in the absence of inhibitors.

1. Ammonia (average pH of 8.03)
2. Ammonia (average pH of 7.91)
3. Nacconol NR
4. Butyl maleimide
5. Emulsol 607

There was no evidence that lipolytic rate was increased by any of the treatments. Emulsol 607 and butyl maleimide had no effect whatsoever. Ammonia had an inhibitory effect similar to that reported in a previous publication (**13**). Nacconol NR, and to a much lesser extent 2′-methyl-l-maleanil,[1] also reduced the rate of lipolysis.

[1] The curve representing lipolysis as a function of length of storage for the 2′-methyl-l-maleanil-treated seeds was not of the usual autocatalytic type, and, therefore, did not lend itself to the same type of kinetic analysis as did the curves for other normal or treated seeds. Nevertheless, a qualitative examination of data from storage of the seeds

Comparison of effects of inhibitor on respiration and lipolysis

Effects of the various treatments on respiration and lipolysis are compared in table II. Ammonia treatment produced consistent changes with

TABLE II

EFFECT OF VARIOUS CHEMICAL AGENTS ON THE RESPIRATION INTENSITY AND LIPOLYSIS RATE CONSTANT OF COTTONSEED

CHEMICAL AGENT	RESPIRATION	LIPOLYSIS
	% of normal	*% of normal*
Ammonia (average pH 8.03)	83	52
Ammonia (average pH 7.91)	38	29
Nacconol NR	169	55
Butyl maleimide	261	100
Emulsol 607	185	100
2′-Methyl-1-maleanil	145	Inhibited

respect to both processes, whereas the other treatments uniformly stimulated respiration, but either inhibited or had no effect on lipolytic activity.

Effect of ammonia concentration on lipolysis

A comparison of the results of the two ammonia treatments indicates that the extent of inhibition of lipolysis is a function of the concentration of the ammonia. This fact is clearly demonstrated in the following series of experiments conducted on cottonseed of the "Cleve Wilt" variety. Three-pound lots of seeds were conditioned to a moisture content of approximately 14.2 per cent. and set aside in air-tight bottles. One portion was used as the untreated control. A second portion was subjected to continuous ammonia treatment by placing in the bottle a small beaker full of ammonium carbonate which slowly decomposed and provided a constant supply of ammonia. A third portion was given only one initial ammonia treatment. A fourth portion was given an initial ammonia treatment, and then stored in an air-tight bottle for 30 days. At the end of this period, the ammonia was removed by withdrawing the seed samples from the bottle and spreading them in a current of air until the excess ammonia was dissipated. The seeds were then replaced in the original bottle and stored together with the other three lots.

The results of this experiment, together with those of certain other experiments to be described later, are shown in table III. The free fatty acid content of two of the ammonia-treated samples remained essentially unchanged for the first 100 days of storage. The third ammonia-treated sample (Lot 405eCr) showed a slight increase in free fatty acids (0.30%) during that period. Marked differences among the samples began to develop during the remaining 230 days of storage. The values of the lipolysis

treated with 2′-methyl-1-maleanil indicated that the rate of free fatty acid formation was definitely inhibited. At the end of 340 days' storage, the sample treated with 2′-methyl-1-maleanil developed 12.5 per cent. free fatty acids, as compared to 17.5 per cent. developed by an untreated control of the same moisture content.

rate constant, k, given in table III represent the overall rates for the entire storage period. It is clear that to realize the maximum effect of inhibition by treatment with ammonia it is necessary to continuously subject the seeds to the action of ammonia vapors.

Nacconol NR was previously found to be the only one of the agents tested which inhibited the lipolytic activity of the cottonseed to a very marked extent. Accordingly, another experiment (405eNv) was planned to determine whether the biological activity of Nacconol NR was due to direct contact with the seeds or to the action of vapors emanating from this material. Three pounds of cottonseed, previously conditioned to 14 per cent. moisture content, were stored in a desiccator above a dish containing 60 gm. of Nacconol NR. In order to compare the activity of solid Emulsol

TABLE III

EFFECT OF INHIBITORS ON THE LIPOLYSIS RATE OF "CLEVE-WILT" VARIETY COTTONSEED

EXPERIMENT	INHIBITOR	MOISTURE CONTENT	FREE FATTY ACID CONTENT			LIPOLYSIS RATE CONSTANT, K	INHIBITION
			ORIGINAL	100 DAYS	230 DAYS		
		%	%	%	%	*reciprocal of days*	%
405 e	None	14.5	0.83	7.14	40.30	20.4×10^{-5}	....
405 eCC	Continuous ammonia	14.7	0.83	0.65	1.78	3.4 "	83.3
405 eC	Single ammonia treatment	14.3	0.83	0.85	7.14	9.6 "	52.9
405 eCr	Excess ammonia removed	14.2	0.83	1.13	28.35	16.7 "	17.9
405 eNv	Nacconol NR vapors	14.0	0.83	1.46	2.75	5.5 "	73.0
405 eE	Emulsol 607M	14.2	0.83	7.21	39.99	20.4 "	None

607M with the previously determined action of an aqueous solution of Emulsol 607, a second experiment (405eE) was conducted by mixing and storing 3 pounds of seed and 10 gm. of Emulsol 607M. From the results given in table III, it is clear that vapors emanating from Nacconol NR have powerful inhibiting properties. On the other hand, Emulsol 607M, a more powerful germicide than Emulsol 607, exhibits no inhibiting effect on the lipolysis rate.

The discovery that vapors from Nacconol NR preparations inhibit the formation of free fatty acids in cottonseed may be of great practical significance to the cottonseed industry. Preliminary mill-scale experiments on the use of this material to improve the storage properties of cottonseed and flaxseed have indicated that improvement may, in fact, be achieved (**2**). Further investigations designed to determine the nature and mode of action of the biologically active vapors are in progress.

Effect of inhibitors on the spectrum of extracted oils

The observation, reported in a previous publication (**1**), that ammonia treatment of cottonseed reduces the color of the solvent-extracted oils was confirmed and extended in the present investigation. It was found, however, that the inhibitors other than ammonia which were tested did not affect the spectrum of the oil. In table IV are shown the results of the effects of various inhibitors on the light absorption of the solvent-extracted cottonseed oils at 560 mμ, the wavelength of maximum light absorption of gossypurpurin (**5**). It can be seen that the concentration of ammonia influences the rate at which the absorption at 560 mμ is reduced. A similar effect was observed when the light absorption of the oil from ammonia-

TABLE IV

THE RELATIVE LIGHT ABSORPTION COEFFICIENT AT 560 mμ OF THE OILS FROM "DELFOS" VARIETY COTTONSEED (100 SERIES) AS AFFECTED BY VARIOUS INHIBITORS

INHIBITOR	LENGTH OF STORAGE	LOG I_o/I*	INHIBITOR	LENGTH OF STORAGE	LOG I_o/I
	days			*days*	
None	29	0.018	Ammonia (ave. pH of 8.03)	32	0.012
	120	0.017		90	0.006
	156	0.011		147	0.004
	337	0.028		326	0.007
Ammonia (ave. pH of 7.91)	32	0.014	Nacconol NR	29	0.018
	90	0.009		71	0.018
	147	0.007		157	0.012
	326	0.006		337	0.038
Emulsol 607	29	0.018	Butyl maleimide	29	0.018
	71	0.018		71	0.021
	157	0.012		158	0.018
	337	0.013		337	0.017
2′-Methyl-1-maleanil	29	0.018			
	71	0.017			
	158	0.012			
	337	0.017			

* All absorption data are calculated for solutions in carbon tetrachloride containing one ml. of oil in a total of 50 ml. of solution. The absorption cell length was 13 mm.

treated seeds at 360 mμ, the wavelength of maximum light absorption of gossypol, was compared to that of the oil from the untreated control.

Effect of ammonia on immature cottonseed

In the course of previous work (**14, 15**), it was noted that an immature sample of seeds ("Coker's 200, strain 1") behaved entirely differently, with respect to both lipolysis rate pattern and respiration intensity, from all of the other samples investigated. The present experiments show that this difference in behavior is also reflected in the reaction of the seeds to ammonia treatment.

When immature seeds were treated with ammonia to raise the pH of the sample to 8.3, the overall respiratory intensity was greatly reduced, in spite of a marked stimulation of respiration immediately following exposure of the seeds to ammonia vapors. Thus, the overall respiratory intensity of untreated immature seeds (sample 204b) was 0.14 cc. of carbon dioxide per gram per day, compared to 0.0027 cc. for comparable ammonia-treated seed (sample 204c). The moisture contents of the two lots were practically the same; *i.e.*, 12.5 per cent. for sample 204b and 12.1 per cent. for sample 204c. The decrease in respiratory intensity was much greater than that usually encountered when mature seeds were treated with ammonia. For example, mature ammonia-treated seeds ("Delfos" variety) which were kept at an average pH of 8.03 had a respiratory intensity of 0.07 cc. of carbon dioxide per gram per day compared to a value of 0.37 for the untreated control lot of the same moisture content. This represents an inhibition of 81 per cent. as compared to an inhibition of 98 per cent. occasioned by similar treatment of immature seeds. In addition to the quantitative differences in respiration intensity between the immature and normal seeds, the respiratory quotient of the ammonia-treated immature seeds (sample 204c) was found to be 0.88 as compared to 0.95 for untreated immature seed (sample 204b). On the other hand, ammonia was found to exert no effect on the respiratory quotient of mature seeds.

TABLE V

CHANGES IN LIGHT ABSORPTION AT 360 mμ AND 560 mμ OF THE OILS EXTRACTED FROM IMMATURE SEEDS (SAMPLE 204) DURING STORAGE

SAMPLE								
204A			204B			204C		
TREATMENT								
NONE			MOISTURE CONTENT REDUCED			AMMONIA		
MOISTURE CONTENT								
13.7 PER CENT.			12.5 PER CENT.			12.1 PER CENT.		
LENGTH OF STORAGE	LOG I_o/I 360 mμ	LOG I_o/I 560 mμ	LENGTH OF STORAGE	LOG I_o/I 360 mμ	LOG I_o/I 560 mμ	LENGTH OF STORAGE	LOG I_o/I 360 mμ	LOG I_o/I 560 mμ
days			*days*			*days*		
0	12.60	0.030	0	8.24	0.041	0	3.19	0.078
41	6.74	0.034	31	4.42	0.031	31	2.59	0.062
69	4.72	0.036	67	3.95	0.033	53	2.16	0.062
112	3.51	0.041	110	2.94	0.048	87	1.96	0.077
142	3.44	0.051	140	3.78	0.048	138	0.74	0.079
212	4.46	0.070	210	6.43	0.074			

Whereas the respiration of immature cottonseed was inhibited by ammonia, this treatment had the opposite effect on the rate of lipolysis. As was shown in figure 5 of a companion paper (**15**), the lipolysis patterns of immature and mature seeds differed fundamentally; the results obtained on immature seeds could not, therefore, be subjected to the same type of mathematical analysis as was used in the case of the results obtained on mature seeds. Both the ammonia-treated and untreated immature seeds gave a similar type of lipolysis pattern. Ammonia stimulated lipolysis of immature seeds, whereas it invariably inhibited lipolysis of the mature seeds. After 100 days of storage, the untreated immature seeds with a 12.5 per cent. moisture content developed 16 per cent. free fatty acids; but after the same period, the ammonia-treated immature seeds with a 12.1 per cent. moisture content developed 24.5 per cent. free fatty acids.

The effects of ammonia treatment on the absorption spectra of oils extracted from immature seeds and on those extracted from mature seeds were also profoundly different. In table V are given the light absorptions at 360 mμ and 560 mμ of the oils from the three lots of immature seeds as a function of length of storage. In table VI is given a summary of all of the types of spectral changes encountered. The untreated immature seeds exhibited at 560 mμ a pattern of change which was not of the same nature found for the mature seeds. Thus, except for an initial drop, the oil from sample 204b exhibited a steady rise in light absorption as the length of the storage period increased. Sample 204a exhibited a steady rise in light absorption from the very beginning of the storage period. On the other hand, the ammonia-treated immature seeds (sample 204c) had an initially higher light absorption in this region, and this absorption did not vary throughout the storage period. The ammonia treatment of mature seeds has been previously shown to lower gradually the light absorption of the extracted oil in the same region (**15**).

TABLE VI

EFFECT OF STORAGE ON THE PATTERN OF CHANGES IN LIGHT ABSORPTION OF THE OILS EXTRACTED FROM UNTREATED AND AMMONIA-TREATED, MATURE AND IMMATURE COTTONSEED

VARIETY	TYPE	TREATMENT	PATTERN OF CHANGES AT 360 mμ	PATTERN OF CHANGES AT 560 mμ
"Delfos"	Mature	None	Reversal of change	Reversal of change
		Ammonia	Gradual drop	Gradual drop
"Coker's-200 strain 1"	Immature	None	Reversal of change	Steady rise
		Ammonia	Gradual drop	No change

With respect to absorption in the 360 mμ region, the two untreated immature seed samples exhibited a reversal of change, whereas the ammonia-treated immature sample showed the steady decrease which is characteristic of treated mature seeds. Thus ammonia treatment has a similar effect on the spectra of the oils of both mature and immature seeds in the 360 mμ region.

The data in table VI confirm the observation made by BOATNER (**5**) that the light absorptions at 360 mμ and at 560 mμ are not due to the same pigment systems. In the mature seeds, these pigments are affected similarly by both storage and ammonia treatment. In immature seeds, however, these two pigment systems behave independently of each other.

Discussion

The initial stimulation of respiration, observed when cottonseed is treated with ammonia, is common to many types of reactions involving poisons and drugs. IRVING (**12**) observed that the respiration of leaves was initially stimulated by exposure to chloroform vapors; when the concentration of chloroform was raised sufficiently, this initial stimulation was rapidly followed by an inhibition of respiration. The initially higher respiration which resulted from the treatment of seeds with ammonia was of such a short duration that the overall effect of this treatment was a decrease in the respiratory intensity of the seeds. It is conceivable that had the concentration of ammonia in the seeds been kept sufficiently low throughout the storage period, the stimulation would have been maintained throughout, and the net effect would have been an increase in respiratory intensity over that of the normal untreated seeds.

An increase in respiratory intensity is what actually occurred in the seeds treated with Nacconol NR, Emulsol 607, butyl maleimide, and 2′-methyl-l-maleanil. In every case, there was a stimulation of respiration which persisted throughout the storage period and resulted in a higher than normal value for respiratory intensity. In large-scale storage experiments, however, it has been possible to increase the concentration of inhibitor (Nacconol NR vapors) to such an extent that the initial stimulation of biological activity is soon replaced by marked inhibition (**2**).

It is significant that the inhibitions of respiration and of lipolysis are not strictly parallel phenomena. That it is possible to inhibit one activity and stimulate the other in the same lot of seeds was shown most clearly by the effect of the Nacconol NR treatment. Thus, it would seem that the processes of respiration and lipolysis are not functionally related in resting seeds; one system may be inhibited under conditions that will stimulate the other. The system involved in lipolysis is apparently more easily inhibited than is that involved in respiration; this is evident in the results obtained on mature seeds where it can be seen there are a number of chemical treatments which inhibit lipolysis but stimulate respiration.

The lack of parallelism between respiration and lipolysis suggests a possible method for distinguishing between deterioration caused by the bacteria and molds that are associated with the seeds and that resulting from the operation of the enzyme systems of the seed themselves. It has been tacitly assumed in many investigations of the deterioration of seeds during storage, that the micro-organisms associated with the seed are the primary cause of deterioration. Thus RAMSTAD and GEDDES (**19**) have suggested that most of the heating that takes place in moist soybeans is due to the action of micro-organisms. Similarly, deterioration in flaxseed has been attributed to microbial activity (**16, 20**). Even though surface sterilization of the seeds rarely affected their rate of respiration or heating, results obtained by use of such procedures could not be offered as clear-cut evidence that the respiration was independent of bacterial action, because surface sterilization obviously could have no effect on the micro-organisms present in the interior of the seeds. Any attempt to achieve sterilization within the seeds would, of course, yield inconclusive results because biological systems of the seeds would also be affected by such a sterilization procedure. Yet, if micro-organisms were the sole cause of respiration, heating, and lipolysis obtained in stored seed, it would be expected that the inhibition of the growth of such organisms would affect all of these processes in a similar manner. On the other hand, if most of the deteriorative action were due to systems within the seeds themselves, it is conceivable that certain biological functions would be more susceptible to inhibition than others. Therefore, when these criteria are applied to the behavior of cottonseed and are considered in conjunction with the evidence presented in this and the preceding papers, it becomes abundantly clear that the bulk of the deteriorative processes in stored cottonseed, such as respiration, heating, and lipolysis, are due to the activity of seed enzyme systems.

The evidence upon which this conclusion is based may be summarized as follows:

1. Surface sterilization has no effect on the respiration of cottonseed. Despite the variety of the fungicides and bactericides used, none has been found to inhibit respiration. These results confirm those of MALOWAN (**17**) who showed that solutions of mercuric chloride or copper sulfate had no inhibitory effect on the heating of cottonseed. Obviously, therefore, surface growth of micro-organisms does not materially contribute to the heating or respiration of the seeds.

2. Nacconol NR has a differential effect on the respiration and lipolysis of cottonseed; under the experimental conditions that were generally used, respiration was stimulated, whereas lipolysis was markedly inhibited. With the exception of the ammonia treatment, the chemical treatments applied stimulated respiration, but they had no such effect on lipolysis.

3. Under certain conditions, it was possible to effect by chemical treatment an inhibition of respiration but a stimulation of lipolysis. Thus, the treatment of immature seeds with ammonia resulted in greatly reduced respiration, but increased lipolysis.

4. Every aspect of the behavior of immature seeds (the 204 series) differs from that of mature seeds; respiration is much higher than normal; the lipolysis pattern is different; spectral changes and the reaction to inhibitors differ markedly from those of normal seed. It is inconceivable that the microbial population of immature cottonseed should differ so greatly from that of mature cottonseed as to change both the degree and pattern of respiration, lipolysis, and spectral changes.

It is possible to conclude, therefore, that under the experimental conditions of temperature and moisture reported in this series of publications, the major portion of the biological activity observed results from the activity of seed enzyme systems. The initial stages of deterioration including the initial heating caused by respiration (**1, 2, 18**), which occur in cottonseed during commercial bulk storage, are almost certainly a result of seed enzymatic activity. Unquestionably, the microbial growth which takes place during storage may reach such proportions as to contribute to the apparent biological activity of the seeds. But this microbial effect is secondary in nature and is evident only after prolonged storage.

It follows from these conclusions that, with the exception of seeds having excessively high moisture content, any attempt to reduce the biological activity of stored cottonseed must concern itself first with the inhibition of the seed enzyme systems.

Summary

1. Treatment of cottonseed with ammonia was found to inhibit the respiration and lipolysis of mature seeds and reduce the light absorption of the extracted oil at 360 mμ.

2. Similar treatment of immature cottonseed was found to inhibit respiration, but to stimulate lipolysis. Light absorption of the extracted oil at 560 mμ was increased, whereas the oil from ammonia-treated mature seeds exhibited decreased absorption at this wave length.

3. The vapors of Nacconol NR were found to inhibit lipolysis in cottonseed under conditions where there was a stimulation of respiration. Treatment of cottonseed with 2′-methyl-l-maleanil yielded similar results.

4. Fungicides and germicides such as Emulsol 607M, Emulsol 607, and butyl-maleimide had no effect on the lipolysis rate of stored cottonseed; the last two substances stimulated respiration.

5. Evidence has been presented to demonstrate that most of the deterioration which occurs in stored cottonseed is due to the action of enzymes in the seeds rather than to microbial activity.

The authors wish to thank G. W. Irving, Jr. and F. G. Dollear for many helpful suggestions. The samples of Nacconol NR, butyl maleimide, and 2′-methyl-l-maleanil were kindly furnished by Lawrence H. Fleet, of the National Aniline Division of the Allied Chemical and Dye Corporation, and the samples of Emulsol 607 and Emulsol 607M were furnished through the courtesy of Albert K. Epstein of the Emulsol Corporation.

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