# COMPARISON OF RESPIRATION, FREE FATTY ACID FORMATION, AND CHANGES IN THE SPECTRUM OF THE SEED OIL DURING THE STORAGE OF COTTONSEED

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(WITH FIVE FIGURES)

## Introduction

The phenomenon referred to as seed deterioration is a complex of a number of different biological and chemical processes. Although a number of these processes have been independently investigated, there is little available information on the relationships existing between them. For example, while it is known that increased moisture content results both in increased lipolytic and respiratory activity, it has not been established whether these two activities are independent manifestations of increased moisture content or whether they are functionally related. For this reason it was of interest to follow lipolytic activity and changes in the seed pigments in those seed samples used for the respiration measurements reported in the preceding publication of this series (**7**).

## Materials and methods

The 3-pound lots of seeds that had been set aside for the respiration measurements (**7**) were used as a source of material for free fatty acid determinations. One portion of the seed oils extracted from samples periodically withdrawn from containers was analyzed for free fatty acid content, while spectrophotometric measurements were made on another portion. The free fatty acid analyses were carried out according to the method previously described by KARON and ALTSCHUL (**6**), and the spectrophotometric measurements as described by ALTSCHUL and coworkers (**1**).

In all that follows, moisture content is expressed on the basis of the total wet weight of the seed, and the amount of free fatty acids in terms of percentage of oleic acid. Spectrophotometric measurements were conducted in a Coleman double monochromator spectrophotometer with an absorption cell thickness of 13 mm.; absorption data refer to a solution containing 1 ml. of oil in a total volume of 50 ml., carbon tetrachloride being used as the solvent.

## Free fatty acid formation

In a previous publication (**6**), it was shown that the rate of lipolysis in cottonseed can be evaluated by the use of the autocatalytic-type equation:

$$dF/dt = k(F)(100 - F) \qquad (1)$$

where $F$ = the percentage of free fatty acids formed,
$100 - F$ = the percentage of residual unhydrolyzed fat,
$t$ = the number of days of storage.

Thus the numerical value of the rate constant, k, in equation (1) quantitatively characterizes the course of free fatty acid formation in cottonseed.

From the data obtained by periodic free fatty acid analyses upon 18 lots of seed, values of the rate constant, k, were determined (table I). It will be noted that there is no evaluation of k for three of the seed lots. The rate of formation of free fatty acids in lot 205a was too low to be accurately measured. The seeds in lots 204a and 204b gave hydrolysis patterns which

TABLE I

THE EFFECT OF MOISTURE ON THE RATE CONSTANT, K, FOR THE FORMATION OF FREE FATTY ACIDS IN STORED COTTONSEED

| SAMPLE | VARIETY | DATE OF HARVEST, 1942 | MOISTURE CONTENT | MOISTURE TREATMENT | K |
|---|---|---|---|---|---|
| | | | % | | *reciprocal of days* |
| 104b | Delfos 3506 | Aug. 29 | 10.0 | Lowered | $1.7 \times 10^{-5}$ |
| 105b | " | Oct. 3 | 10.6 | None | 2.2 " |
| 104a | " | Aug. 29 | 12.0 | None | 2.0 " |
| 105a | " | Oct. 3 | 13.1 | Raised | 5.0 " |
| 105f | " | " | 14.2 | Raised | 8.7 " |
| 105f′ | " | " | 14.9 | Raised | 13.6 " |
| 105e | " | " | 16.8 | Raised | 17.6 " |
| 205a | Coker's 200, strain 1 | Sept. 22 | 12.5 | Raised | ......... |
| 204b | " | " | 12.5 | Lowered | ......... |
| 204a | " | " | 13.7 | None | ......... |
| 206a | " | Oct. 22 | 14.7 | Raised | 6.0 " |
| 205e | " | Sept. 22 | 15.9 | Raised | 13.6 " |
| 305b | Oklahoma Triumph, 0–52–12 | Oct. 1 | 10.6 | None | 1.4 " |
| 306b | " | Oct. 28 | 10.6 | None | 2.4 " |
| 307a | " | Nov. 28 | 12.9 | Raised | 3.2 " |
| 306a | " | Oct. 28 | 13.1 | Raised | 2.6 " |
| 304a | " | Sept. 22 | 13.3 | Raised | 3.6 " |
| 308a | " | Dec. 28 | 15.7 | None | 12.9 " |

were fundamentally different from those of the other seed samples investigated.

The values of k are plotted as a function of moisture content in figure 1. The experimental points for the samples in the 100 series all fall on one curve, whereas the points for the samples in the 200 and 300 series fall on a second curve which is lower than the first. In this respect the results of the lipolysis rate determinations resemble very closely those of the respiration measurements on the same seed sample (7), since in both instances a higher level of activity at comparable moisture contents was exhibited by the seeds in the 100 series.

The curves in figure 1 lend themselves to further analysis by the use of the following autocatalytic-type equation:

$$dk/d(H_2O) = k'k(A - k) \qquad (2)$$

where $k$ = lipolysis rate constant,
$(H_2O)$ = percentage moisture in the seed,
$k'$ = constant relating moisture content to lipolysis rate constant
$A$ = arbitrary constant.

Upon integration, equation (2) becomes

$$\log k/A - k = A\,k' \quad (H_2O)/2.3 + \text{constant}. \qquad (3)$$

If the equation is applicable to the curves in figure 1, there should exist a value of A for which a plot of $\log k/A - k$ against $(H_2O)$ will give a straight

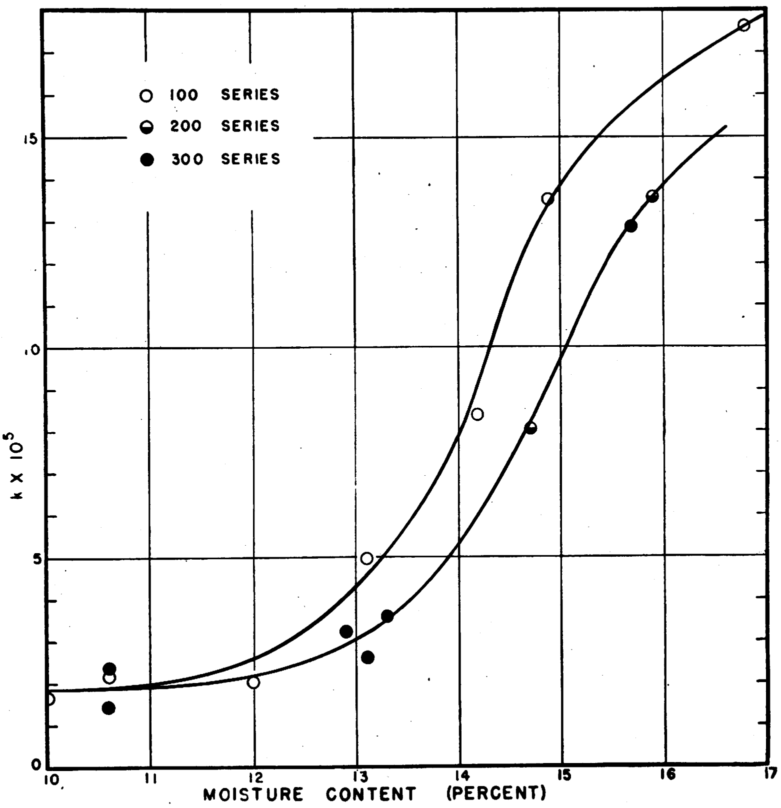


FIG. 1. The effect of moisture content on the lipolysis rate constant of cottonseed.

line with a slope equal to $A\,k'/2.3$. When a value of $50 \times 10^{-5}$ was chosen for A, it was found that straight lines could be obtained to represent in a linear manner the effect of moisture on a function of the lipolysis rate constant. As is shown in figure 2, the data for the seeds in the 100 series fall on one straight line in the range of 11–16 per cent. moisture and the data for the seeds in the other two series fall on a second straight line. In order to compare the properties of the present seed samples with those used in a previous investigation (**6**), the rate constants for these seed samples were treated in the same manner and here again a straight line was obtained[1] (curve 1, figure 2). The slopes of the lines in figure 2 are 0.27 per one per cent. moisture for the seeds in the 200–300 series, 0.33 for those in the 100 series, and 0.43 for the "Delfos" (**6**) seeds harvested in 1941.

It is interesting to note that the methods of analysis used to express respiration intensity and rate of hydrolysis as linear functions of the mois-

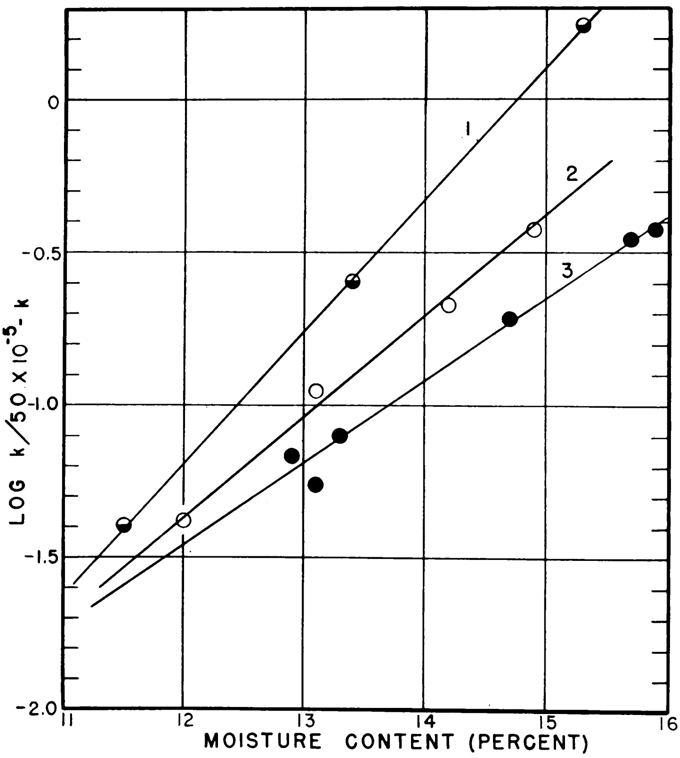


FIG. 2. The effect of moisture content of cottonseed on a function of the lipolysis rate constant.

1. "Delfos" variety of cottonseed (**6**), harvested in 1941.
2. 100 series of seeds, "Delfos" variety harvested in 1942.
3. 200 and 300 series of seeds, "Coker's" and "Oklahoma Triumph" varieties, harvested in 1942.

ture content indicate different types of relationship between seeds in the 100 series and the 200 and 300 series. From the respiration data it was found that the seeds in the 200 and 300 series exhibited the same sensitivity to increase in moisture as did the seeds in the 100 series, manifested by the fact that the slopes of the two lines representing these series were the same (**7**). The two series differed, however, in that the 100 series respired at a

[1] In order to compare previous results (**6**) with those reported in this investigation, it was necessary to convert the values for the percentage moisture content from the dry basis used previously to a wet basis as reported in this publication.

higher level of intensity. The hydrolysis data, on the other hand, revealed that the seeds of the 100 series exhibited a higher level of lipolysis and also displayed a higher sensitivity to increased moisture than those in the 200 and 300 series. In both lots practically the same rate of lipolysis occurred at a moisture content of 11 per cent., but great differences were observed at higher moisture values.

It is not immediately apparent whether this difference between the rate of respiration and lipolysis represents an intrinsic biological phenomenon or whether the relationship that seems to exist is a result of the methods

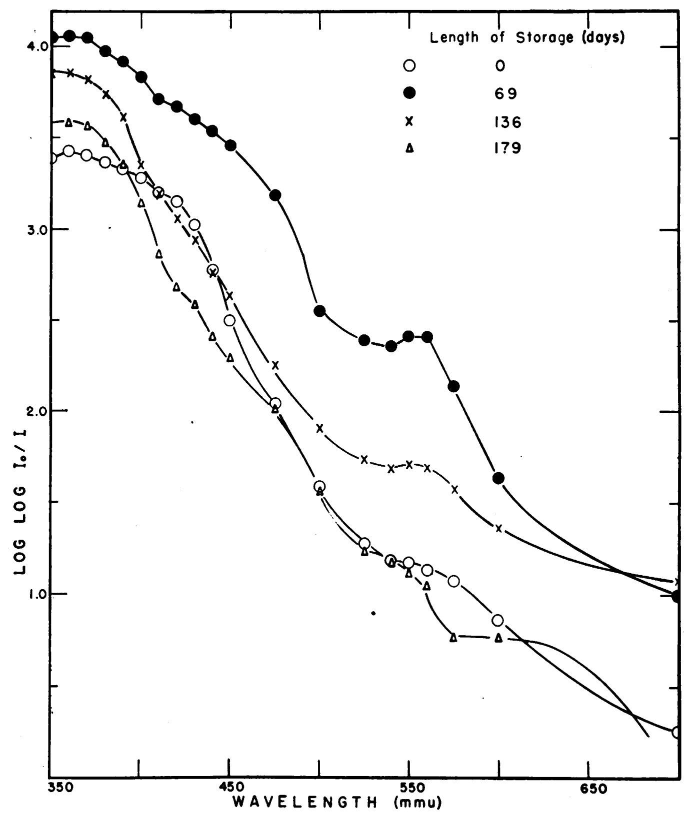


FIG. 3. The variation in the absorption spectrum of extracted oils from sample 205e during storage.

of mathematical analysis used. For all practical purposes the respiratory intensity, defined as the average respiration rate of the seed, is independent of the character of the respiratory pattern. On the other hand, the hydrolysis-rate constant, k, is determined by the rate of increase in free fatty acids in the seeds as a function of the length of storage. It is quite possible, therefore, that two such different constants would not respond in the same manner to mathematical analysis. Unfortunately, because of the irregular nature of the respiratory patterns, it is not possible to use any but an averaging process to arrive at a quantitative value for respiratory intensity. On this basis there cannot be obtained a mathematical measure of the sensitivity of respiratory intensity to moisture which will be wholly comparable to that for lipolysis.

### Absorption spectra of solvent-extracted oils

When cottonseed flakes or meats are extracted with fat solvents, there are extracted along with the oil a considerable quantity of pigments whose nature has been the subject of many investigations. As a result of such investigations, a new class of pigments peculiar to cottonseed has been disclosed. Of these, gossypol, a yellow pigment, is the most abundant and the best known. BOATNER *et al.* (**3, 4**) have shown, however, that at least four pigments besides gossypol exist in cottonseed and that some of these, at least, are interrelated.

TABLE II

THE RELATIVE LIGHT ABSORPTION COEFFICIENT AT 560 mμ OF THE OILS FROM THE COTTONSEED SAMPLES IN THE 100 SERIES

| SAMPLE | LENGTH OF STORAGE | LOG $I_o/I$ | SAMPLE | LENGTH OF STORAGE | LOG $I_o/I$ |
|---|---|---|---|---|---|
| | *days* | | | *days* | |
| 104b | 0 | 0.012 | 104a | 0 | 0.021 |
| | 87 | 0.010 | | 52 | 0.012 |
| | 232 | 0.019 | | 115 | 0.007 |
| | 367 | 0.032 | | 218 | 0.014 |
| | | | | 367 | 0.037 |
| 105a | 0 | 0.005 | 105b | 0 | 0.011 |
| | 27 | 0.009 | | 71 | 0.010 |
| | 122 | 0.011 | | 160 | 0.008 |
| | 328 | 0.021 | | 342 | 0.026 |
| 105f | 0 | 0.013 | 105f′ | 29 | 0.018 |
| | 66 | 0.015 | | 120 | 0.017 |
| | 120 | 0.016 | | 156 | 0.011 |
| | 337 | 0.016 | | 337 | 0.028 |
| 105e | 27 | 0.036 | | | |
| | 62 | 0.032 | | | |
| | 111 | 0.012 | | | |
| | 147 | 0.020 | | | |
| | 223 | 0.093 | | | |

Much of the pigmented material in the cottonseed is localized in structures known as pigment glands distributed throughout the embryo (**8**). All of the pigments so far isolated have been found in these glands, from which they can be readily extracted by use of suitable solvents (**5**). There are solvents, however, that are incapable of breaking up the glandular structure. These solvents are able to extract only those pigments that occur outside the glands or are contained in the small number of ruptured glands present in cottonseed flakes or ground cottonseed. An example of solvents of the latter type is Skellysolve F (low-boiling petroleum ether).

In the experiments reported above, petroleum ether was used to extract

## TABLE III

THE RELATIVE LIGHT ABSORPTION COEFFICIENT AT 560 mμ OF THE OILS FROM THE COTTONSEED SAMPLES IN THE 200 SERIES

| SAMPLE | LENGTH OF STORAGE | LOG $I_o/I$ | SAMPLE | LENGTH OF STORAGE | LOG $I_o/I$ |
|---|---|---|---|---|---|
| | *days* | | | *days* | |
| 204a | 0 | 0.030 | 204b | 0 | 0.041 |
| | 69 | 0.036 | | 31 | 0.031 |
| | 142 | 0.051 | | 67 | 0.033 |
| | 212 | 0.071 | | 140 | 0.048 |
| | | | | 210 | 0.074 |
| 205a | 0 | 0.044 | 206a | 0 | 0.017 |
| | 29 | 0.025 | | 56 | 0.013 |
| | 179 | 0.025 | | 109 | 0.013 |
| | 338 | 0.064 | | 244 | 0.071 |
| | | | | 316 | 0.043 |
| 205e | 0 | 0.014 | | | |
| | 69 | 0.258 | | | |
| | 136 | 0.048 | | | |
| | 179 | 0.011 | | | |

the seed oil which was then analyzed for free fatty acids. On aliquots of the same extracted oil, periodic spectroscopic determinations were also made. As an example of the type of results obtained in the spectroscopic analyses, the data for sample 205e are given in figure 3. It will be noted that as storage progresses there is a change in the spectra of the oils. At 560 mμ, the point of maximum light absorption of the pigment gossypurpurin (**3**), there is first an increase in absorption, which is followed by a decrease as the storage is continued. The light-absorption value after 179 days of storage is very close to the original value. A similar pattern of

## TABLE IV

THE RELATIVE LIGHT ABSORPTION COEFFICIENT AT 560 mμ OF THE OILS FROM THE COTTONSEED SAMPLES IN THE 300 SERIES

| SAMPLE | LENGTH OF STORAGE | LOG $I_o/I$ | SAMPLE | LENGTH OF STORAGE | LOG $I_o/I$ |
|---|---|---|---|---|---|
| | *days* | | | *days* | |
| 304a | 0 | 0.007 | 305b | 0 | 0.020 |
| | 32 | 0.022 | | 71 | 0.019 |
| | 91 | 0.026 | | 267 | 0.070 |
| | 146 | 0.038 | | 339 | 0.053 |
| | 225 | 0.100 | | | |
| | 327 | 0.105 | | | |
| 306a | 0 | 0.032 | 306b | 0 | 0.018 |
| | 56 | 0.030 | | 68 | 0.043 |
| | 110 | 0.040 | | 323 | 0.068 |
| | 212 | 0.084 | | | |
| | 317 | 0.058 | | | |
| 307a | 0 | 0.022 | 308a | 0 | 0.021 |
| | 64 | 0.023 | | 55 | 0.072 |
| | 144 | 0.043 | | 133 | 0.088 |
| | 282 | 0.079 | | 237 | 0.012 |

change is observed in the absorption at 360 mμ, a point of maximum light absorption of gossypol (**3**). Again there is an increase in the light absorption during the initial stages of storage followed by a decrease as storage is continued.

The effect of storage on the light absorption of the oil at 560 mμ is given in tables II, III, and IV. Inasmuch as it has already been shown that the 200 and 300 series of seeds behave similarly with respect to both their respiration behavior and lipolysis rates, it was of interest to examine the experimental data to determine whether there might be a similar correlation between the

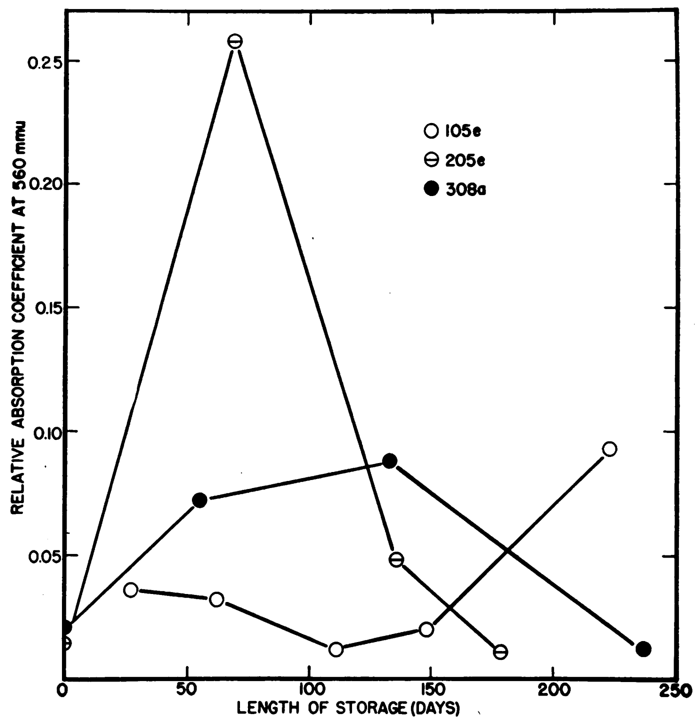


FIG. 4. The effect of storage on the relative light absorption coefficient at 550 mμ in the case of high-moisture seeds.

changes in the light absorption of the oils and other seed characteristics. Accordingly, the relative absorption coefficient at 550 mμ for the sample having the highest moisture content in each series was plotted against the days of storage (fig. 4). It is significant to note that the patterns of change in the 200 and 300 series are similar in that they both exhibit a maximum during the storage period. On the other hand, the pattern in the 100 series is reversed; *i.e.*, there is a minimum in absorption during the same storage interval.

As yet too little is known concerning the chemistry and biochemistry of cottonseed pigments to permit one to draw any significant conclusions from the above-mentioned data. Inasmuch as Skellysolve F does not rupture the pigment glands, the observed changes in light absorption may be due both to changes in the relative pigment concentrations in the seeds and glands and to changes in the amount of Skellysolve F–soluble pigmented material which occurs outside of the glands. As soon as more information concerning cottonseed pigments is available, it should be possible to interpret data of this type with more certainty and to correlate them with other seed characteristics.

## Immature seed

In the preceding publication (**7**) it was indicated that the samples of immature seed, 204a and 204b, exhibited a much higher level of respiration than did any of the other samples. An examination of the course of lipolytic activity in these same seeds indicated that they differed materially also

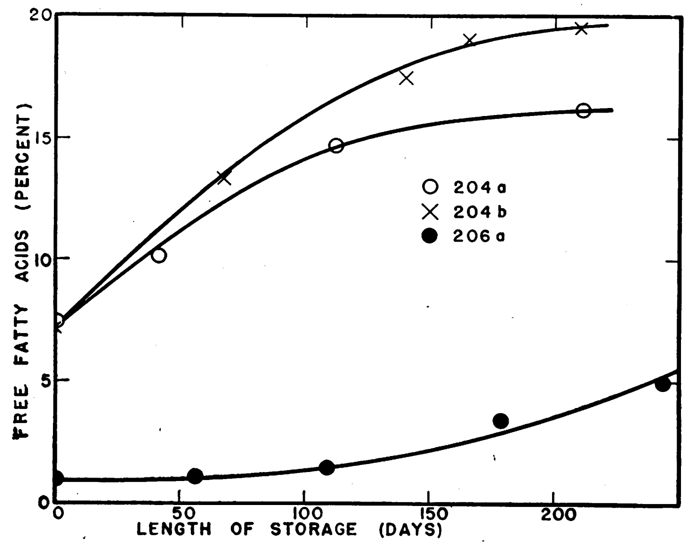


FIG. 5. Free fatty acid formation during the storage of samples 204a, 204b, and 206a.

from the other samples with respect to their rate and pattern of formation of free fatty acids. Whereas all of the other seed samples exhibited a normal accelerating rate of lipolysis as storage continued (**6**), the immature seeds exhibited the highest rate of lipolysis at the beginning of the storage period. As is shown in figure 5, where the curves for the rate of development of free fatty acids in samples 204a and 204b are compared to a curve for normal seeds of about the same moisture content, the rate of formation of free fatty acids in the immature seeds at the end of 200 days' storage was practically zero. On the other hand, the normal seeds were just beginning to display an appreciable rate of lipolysis at that time.

## Discussion

There would appear to be two alternative explanations of the parallel relationship which exists between the vigor as reflected by lipolytic rate and the vigor as reflected by respiratory rate of the three series of seeds investigated: (1) the two processes depend on the same biological systems, or (2) they reflect a general condition in the seeds whereby all types of enzymatic processes are maintained at a similar level of activity. The former explanation, *i.e.*, that lipolysis and respiration are functionally related, is probably not correct, in view of the differential effect of inhibitors on these two processes as shown in another publication (**2**).

It seems evident that the intensity of respiration in seeds is dependent on the amounts of the respiratory and associated enzyme systems that are present in an active state and on the rate of release or production of additional active enzymes (**7**). Similarly, the rate of lipolysis is determined by the initial concentration of active lipases and by the ability of the cell systems to release additional lipolytic enzymes as the storage progresses. The fact that seeds which exhibit equal respiratory activities (the 200 and 300 series) also exhibit equal lipolytic activities is a strong indication that seeds may be characterized by their state of "biological vigor." At least for the two variables, lipolysis and respiration, vigor with respect to one is accompanied by equal vigor with respect to the other. On the basis of this reasoning it is not inconceivable that the seeds in the 200 and 300 series would show similar behavior and lower vigor than those in the 100 series with respect to other properties such as germination, strength, and rate of growth of the seedlings. There is, however, no evidence in the present work which substantiates such a generalization.

That both respiration and lipolysis equally reflect the vigor of cotton seeds is a fact of great practical importance in commercial storage. On the basis of this fact it would be reasonable to predict that seeds whose respiration intensity is high—that is, seeds that heat readily when stored in bulk would, even when heating is controlled by air circulation, undergo more intensive lipolysis than seeds of equal moisture content but lower respiratory activity. Thus, the measurement of the heat resulting from respiration should be a useful criterion in predicting the storage behavior of seeds.

The behavior of immature seeds is entirely different from that exhibited by mature seeds. In immature seeds, the enzyme systems have not reached the state of dormancy which characterizes them in mature seeds. For this reason immature seeds are more sensitive to moisture and respire at a higher intensity than do mature seeds. The course of lipolysis in immature seeds indicates that the hydrolytic activity is due exclusively to enzymes already present in the active state and that no production of additional active enzymes takes place during the course of storage. Actually, it would seem that the enzyme systems responsible for free fatty acid formation are rendered less and less active as the storage of immature seeds progresses. In effect, then, the maturation process which is interrupted by early harvesting of the cotton, is continued during storage.

## Summary

1. Samples of cottonseed that were used in the respiration investigations reported in a previous paper (**7**) were analyzed for free fatty acid content, and the lipolysis-rate constant, k, was determined for each of the samples.

2. When the lipolysis constants were plotted as a function of moisture content, it was found that the constants for the "Delfos" seed all fell on a smooth curve and that constants for the "Coker's" and "Oklahoma Triumph" varieties fell on a second smooth curve which was lower than the "Delfos" curve.

3. It was found that the lipolysis constant can be converted into a linear function of the moisture content of the seed by the use of the differential equation:

$$dk/d(H_2O) = k' \, k \, (A - k)$$

where $k$ = hydrolysis rate constant,
$(H_2O)$ = percentage moisture in the seed,
$k'$ = constant relating moisture content to lipolysis rate constant
$A$ = arbitrary constant.

When A is given the value of $50 \times 10^{-5}$, the log of $k/A - k$ becomes a linear function of the moisture content.

4. The slopes of the lines denoting lipolysis as a linear function of moisture content were found to be 0.27 unit per one per cent. moisture for the "Coker's" and "Oklahoma Triumph" seeds and 0.33 unit for the "Delfos" seeds.

5. A variation of the spectrum of solvent-extracted oils during the storage of cottonseed was found to exist. The highest moisture samples of the "Coker's" and "Oklahoma Triumph" varieties showed a common pattern which was in turn different from that exhibited by "Delfos" variety.

6. The pattern of lipolysis for immature seeds was shown to be different from that for normal cottonseed in that the rate of formation of free fatty acids decreased with length of storage.

7. The "Delfos" seeds exhibited more vigor both with respect to lipolysis and respiration than did the seeds in the "Coker's" and "Oklahoma Triumph" varieties.

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