A RAPID METHOD FOR THE MEASUREMENT OF THE INHIBITION OF DETERIORATION IN INTACT SEEDS

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(WITH EIGHT FIGURES)

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Introduction

In several previous publications (**1, 2, 3, 11**), it was shown that treatment of cottonseed with ammonia and other chemicals prior to storage reduced respiration, heating, and lipolysis in the stored seed. On the basis of these experiments, it was concluded that chemical inhibition of biological activity resulting in deterioration in seeds is possible of accomplishment, and the need for finding new compounds which are active in small concentrations became increasingly important. Heretofore, the number of chemicals assayed has been limited because of the length of time required to test their inhibitory efficiency. For example, the measurement of inhibitory action upon lipolysis in cottonseed at room temperature requires several months under ordinary conditions of storage before conclusive results can be obtained. A quantitative evaluation of the efficiency of an inhibitor, on the same basis, requires numerous tests extending over a period of at least a year. In order to survey a wide variety of chemicals for inhibitory efficiency it was necessary to devise a method for rapidly evaluating a large number of the potential inhibitors simultaneously, and making accurate quantitative measurements of the relative efficiency.

Two prerequisites of an inhibitor capable of arresting deterioration during storage of seeds intended for processing are: (1) that it minimize biological activity leading to deterioration and, (2) that it preserve the quality of the derived products. In order to evaluate a chemical rapidly with respect to these two properties, experimental conditions must be applied in the laboratory which will approximate those existing during commercial storage, and which will permit quantitative evaluation of both biological activity and the quality of the product after storage. In the case of cottonseed, a method which measures the effectiveness of an inhibitor with respect to heating and lipolysis satisfies the above criteria.

Moist cottonseed when stored in bulk will heat relatively rapidly and temperatures as high as 175° F. have been recorded in such seed (**13, 14**). High temperatures have also been recorded during the storage of other types of seeds (**8, 9, 12, 17, 19**). A number of investigators have attributed the heating and deterioration of grain during storage to the organisms associated with the grain (**12, 15, 16**). Such deterioration may be equally as well ascribed to the activity of the enzymes in the seeds themselves (**5**). It

cannot be overlooked that the enzymes in the seeds and those in the microorganisms may be responsible in different degrees for the biological activity of the seeds. From the results presented in this paper it is not possible to conclude definitely what relative role is played by the enzymes from the two sources.

The product of prime economic importance derived from cottonseed is the oil, the quality of which is expressed in terms of refining loss, color, odor, and flavor. Extensive deterioration in cottonseed results from lipolysis which is reflected in a high refining loss and consequently a low quality of crude oil.

With other oilseeds such as soybeans, flaxseed, and sunflower seed, the procedure outlined for cottonseed may be used. For other classes of seeds, another measure of quality of the product which is characteristic of the seed can be substituted for the measurement of lipolysis. LARMOUR (**12**), for example, used a baking test to evaluate the effect of an inhibitor on the quality of the flour milled from stored wheat.

The rapid method of assaying inhibitors which has been mentioned in a preliminary report (**10**) and which is here described in detail embodies the following steps:

1. Conditioning of prime seed to a sufficiently high moisture content to promote rapid heating and deterioration.
2. Treating of a portion of the conditioned seed with the desired quantity of potential inhibitor.
3. Storing samples of the treated and untreated seed in calorimeters for 6 days under conditions of aeration which will support maximum heating.
4. Recording the temperatures in the seed during the period of storage.
5. Analyzing the seed-oil at the end of the storage period for the percentage of free fatty acids.

By the arbitrary choice of the effect produced by one inhibitor as a standard and by the comparison of the other chemicals to this standard and to the untreated seed of the same moisture content, it was possible to evaluate quantitatively the extent of inhibition or stimulation of biological activity produced by each chemical.

Apparatus

RAMSTAD and GEDDES (**18**) applied an adiabatic respirometer for measuring respiration and heating of soybeans. In this apparatus, heat loss from the calorimeter was minimized by continuously maintaining the temperature of the surrounding air a small fraction of a degree lower than that of the heating seed. This technique was applied in an investigation of spontaneous heating of flaxseed and sunflower seed (**19**). Although application of the adiabatic technique eliminates heat losses and avoids fluctuations resulting from changes in room temperature, this advantage of the adiabatic calorimeter had to be dispensed with in favor of a method which would permit the simultaneous evaluation of a large number of inhibitors.

Dewar flasks of one-liter capacity were used as calorimeters. Because of the variation in the insulating capacity of individual Dewar flasks, each flask was tested before being used. Water at 140° F. was placed in the flask which had been preheated to the same temperature. If the temperature of the water did not decrease more than 25° F. in 21 hours, the insulating capacity of the flask was considered adequate. Periodic checks on the rate of cooling of each flask assured uniformity among the calorimeters.

Figure 1 represents a cross-section of a calorimeter showing the inner fittings which consist of a plastic disk, seated approximately one inch from the floor of the flask; a plastic aeration tube,[1] one-quarter inch in diameter, centered in the cork stopper and the plastic disk, by means of which the

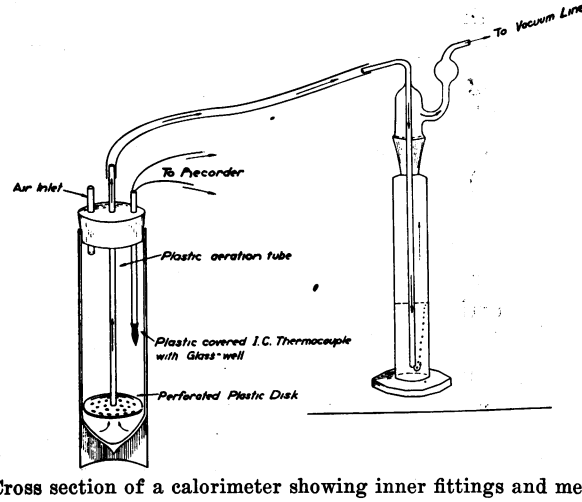

FIG. 1. Cross section of a calorimeter showing inner fittings and method of aerating the seed.

column of seed resting on the disk is aerated; and the plastic-covered, glass-tipped thermocouple which serves as a means of measuring the temperature of the seed. The aeration tube is connected to a gas washing bottle and finally to a vacuum system. The gas washing bottle contains a buffered solution of bromthymol blue, which changes from blue to green to yellow as the total quantity of carbon dioxide passing through the solution increases (**7**). By maintaining the liquid in the gas washing bottle at a specified level, the flow of air through the system can be adjusted to the desired rate. The batteries of calorimeters, aeration devices, recorders, and vacuum system are illustrated in figure 2. A repeating multipoint switch (**4**) was designed and constructed in order to record the temperatures in a number of calorimeters on a single point recorder.

Materials and methods

Flaxseed was used as the experimental material since it is well adapted to routine tests. It has a relatively high density, requires considerably less

[1] Obtained from Extuded Plastics, Norwalk, Conn.

time to reach equilibrium when artificially conditioned to a high moisture content, and is easily mixed with the chemicals to be tested. Two varieties of Texas-grown flaxseed, Rio and Golden Viking, were found equally satisfactory. Lots of prime seed of these varieties were obtained and stored in a refrigerator until used. They were conditioned to the desired moisture content before use.

When water is added to the seed at room temperature, sufficient heat is generated in the first 24 hours to produce typical "hot pockets" of burned seed. On the other hand, if the seed is conditioned at 36° F. and allowed to absorb moisture spontaneously from an atmosphere maintained at 100%

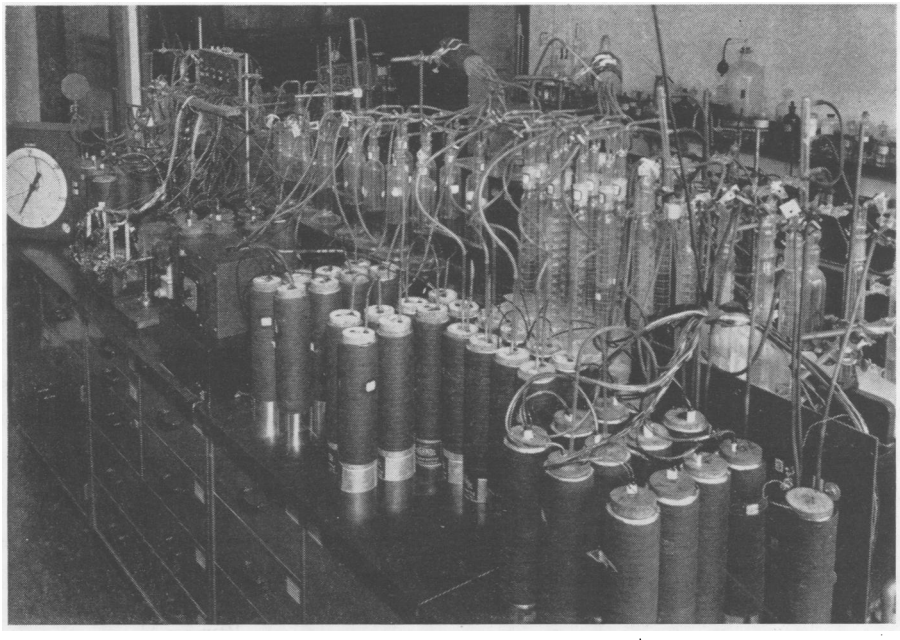

FIG. 2. Battery of calorimeters and aerating devices.

humidity, the absorption of moisture is so slow that by the time the desired moisture content is attained, sufficient deterioration has taken place to render it unfit for use. The seed is therefore cooled to 36° F. and conditioned by adding the calculated quantity of cold (36° F.) distilled water and by maintaining this temperature for the three to five days required by the seed to come into equilibrium. During this period, the seed is mixed each day and care is taken to break up all lumps or aggregates.

On the fourth day a 600-gm. portion of the seed is treated with the required volume or weight of the chemical to be investigated. The chemical is dissolved in a diluent to make a total volume of 10 ml., which is then added to the seed in four aliquots with thorough mixing after each addition. Another 600-gm. portion of the seed is treated with the diluent only and serves as the control. Each sample, treated and control, is divided into two

300-gm. portions which are placed in screw-top jars and stored in a refrigerator at 36° F. for three hours immediately prior to loading into the calorimeters. This procedure allows the samples to be started at a uniform temperature and provides for adequate diffusion of the chemical through the seed by the time that the samples reach room temperature.

Each calorimeter holds 300 gm. of flaxseed allowing approximately two inches of free space between the top of the column of seed and the bottom of the stopper. Each test and control is made in duplicate. The test is concluded in six days after which the seed is removed and examined visually relative to its state of preservation. The sample is then ground and the percentage of free fatty acid is determined on the extracted oil by the method of the American Oil Chemists' Society (**6**). The result is expressed as percentage of oleic acid. The moisture content is determined by the method of the American Oil Chemists' Society (**6**) and reported on a *per se* basis.

Experimental results

Effect of moisture content and aeration

The most favorable conditions for rapid heating in samples of insulated seed are high moisture content and adequate aeration. The effect of moisture on the rate of heating of Golden Viking flaxseed (No. 172) was determined by conditioning several samples to 8, 12, 16, and 20% of moisture and storing them in the calorimeters. The heating curves for these samples are reproduced in figure 3 from which it is evident that in six days there is a rapid rise in temperature if the moisture is sufficiently high.

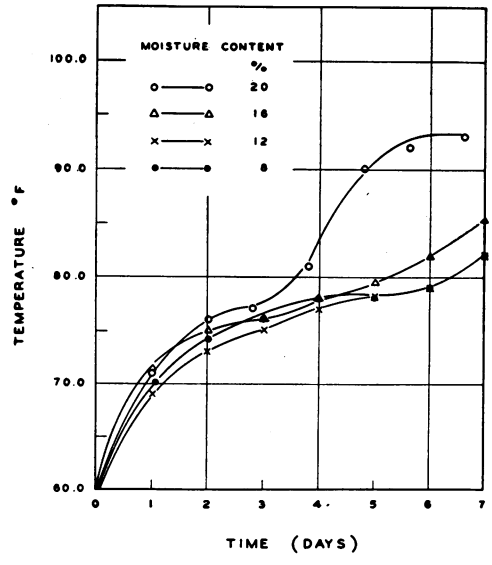

Fig. 3. The effect of moisture content on the rate of heating of Golden Viking flaxseed.

The rate of respiration of the seed is increased by aeration, and because of the relationship between respiration and heating (**12, 15, 16**), it should be expected that aeration would increase the rate of heating. Therefore, if the heating effect due to aeration is superimposed upon that resulting from a high moisture content, a further increase in the rate of heating should follow. Samples of flaxseed (300 gm.) of the Rio variety (No. 173) were artificially conditioned to a moisture content of 22%, and aerated in duplicate at the rate of 3, 10, 15, and 20 liters of air every 24 hours. The temperature of the air was that of the room, averaging 80° F. The effects of the various rates of aeration are shown graphically in figure 4 from which it is apparent that

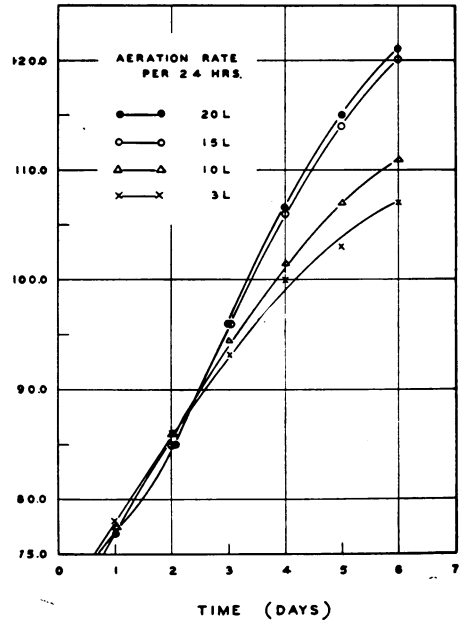

FIG. 4. The effect of varying the rate of aeration (average temperature of air, 80° F.) on a sample of artificially conditioned (22% moisture) Rio flaxseed.

a rate of 15 liters per 24 hours produces the optimum heating in six days.

At the expiration of the six days, the seed was removed from the Dewar flasks and free fatty acid determinations were made on the extracted oils. The average values of quadruplicated determinations are reported in table I. These results indicate that the seed which was aerated at the rate of 15 liters per 24 hours exhibited the maximum hydrolysis of the glycerides in the oil. Since sufficiently high maxima in temperature and lipolysis were exhibited in 6 days by seed containing 22% moisture under aeration at a rate of 15 liters per 24 hours, these conditions were considered adequate to produce rapid deterioration.

EFFECT OF SOLVENT

A comparison of the behavior of flaxseed treated with diethyl ether (10 ml. ether per 600 gm. of seed) to that of untreated seed from the same lot

TABLE I

LIPOLYSIS IN 300-GRAM SAMPLES OF RIO FLAXSEED (NO. 173) ARTIFICIALLY CONDITIONED TO 22% MOISTURE CONTENT AS EFFECTED BY VARIOUS RATES OF AERATION

RATE OF AERATION	FREE FATTY ACID PRODUCTION
Liters per day	%
3	5.3
10	7.3
15	8.2
20	6.4
Refrigerated control*	0.8

* Seeds conditioned to 22% moisture content and stored at 36° F. for 6 days.

and having the same moisture content showed that the ether has no effect on the rates of heating and lipolysis. At this low concentration diethyl ether, therefore, may be used as a vehicle for dispersion of the chemicals to be tested. The activity of a number of organic solvents was compared to that of ether with results given in table II. All of the solvents investigated exhibited some activity with respect to heating or lipolysis or both when applied at the same concentration as diethyl ether and, therefore, cannot be used as diluents.

APPLICATION OF METHOD

ETHYLENE CHLORHYDRIN.—Six-hundred-gram portions of flaxseed (No. 156), conditioned to a moisture level of 22% were treated, respectively, with 2.5, 1.5, and 0.5 ml. of anhydrous ethylene chlorhydrin, dissolved in sufficient diethyl ether to make a total volume of 10 ml. These portions were then divided into duplicate 300-gm. samples and placed in the colorimeters. Seed of the same moisture content was treated with 9.5 ml. of diethyl ether per 600 gm. of sample and used as controls. Figure 5 shows the heating

TABLE II

THE EFFECT OF 10 ML. VOLUMES OF DIFFERENT DILUENTS ON THE HEATING AND LIPOLYSIS OF 600-GRAM PORTIONS OF FLAXSEED (22% MOISTURE) AS COMPARED TO THAT OF DIETHYL ETHER

DILUENT	EFFECT ON	
	HEATING*	LIPOLYSIS*
Diethyl ether	.....	.....
Petroleum naphtha	.....	S
Cyclohexane	I(s)	I(s)
Dioxane	I	I(c)
Acetone	I	I(s)
Ethyl acetate	I(c)	I
Isooctane	.....	S
Chloroform	I	I
Toluene	I	I(s)
Butyl acetate	I	I
Amyl acetate	I	I

* I = inhibition; S = stimulation; (c) = complete; and (s) = slight.

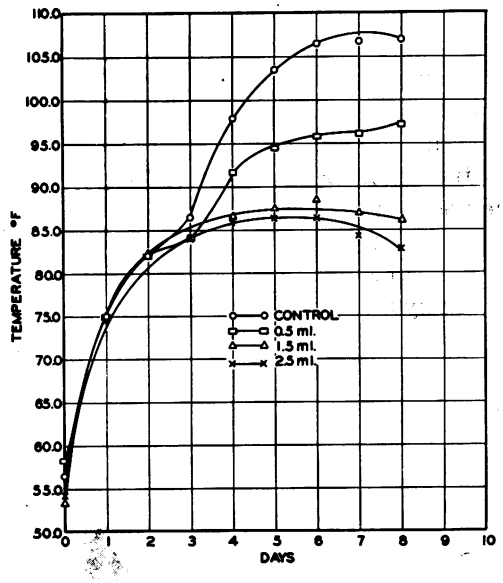

FIG. 5. Inhibition in heating resulting from treatment with various concentrations of ethylene chlorhydrin.

curves for these samples. During the experiment, room temperature remained at 84° F. while that of the untreated control rose to 107.5° F. Increasing concentrations of ethylene chlorhydrin produced increasing degrees of inhibition in heating. The difference between the use of 1.5 and 2.5 ml. of ethylene chlorhydrin was not significant and the percentages of inhibition produced in heating closely approximated 100%. Even the lowest concentration of ethylene chlorhydrin showed some inhibition of heating.

Results of free fatty acid determinations made on the oils extracted from the seed after removal from the calorimeters are recorded in table III. The free fatty acid content of the original unconditioned seed was 0.94%. The

TABLE III

EXTENT OF LIPOLYSIS IN FLAXSEED TREATED WITH ETHYLENE CHLORHYDRIN AND KEPT IN CALORIMETERS FOR EIGHT DAYS

VOL. OF INHIBITOR	CONC.* OF INHIBITOR	FREE FATTY ACIDS†	MOISTURE
ml.	%	%	%
.....	.....	5.03	20.9
2.5	0.64	0.9	21.5
1.5	0.38	1.4	21.2
0.5	0.13	6.3	21.1
Refrigerated control‡		1.1	21.6

* Per dry weight of seeds.
† Initial free fatty acid content is 0.9%.
‡ Flaxseed conditioned to a moisture level of 22% and kept at 36° F. for eight days.

highest concentration of ethylene chlorhydrin completely inhibited lipolysis whereas the seed treated with a concentration of 0.38% (1.5 ml.) of the same compound developed an average of 1.4% of free fatty acids. This degree of inhibition compares favorably with that occurring in the refrigerated untreated control, a sample from the same batch of conditioned seed maintained at a temperature of 36° F. for the eight days of the experiment, during which time the free fatty acid content rose to 1.1%. Although the difference in effectiveness between the 0.38% and 0.64% concentrations is barely significant, when the concentration of inhibitor decreased to 0.13% the quantity of free fatty acids formed increased over and above that of the untreated control indicating a very definite stimulation of lipolysis.

Detailed observations were made when the samples of seed were removed from the Dewar flasks. Some idea of the relative condition of the untreated

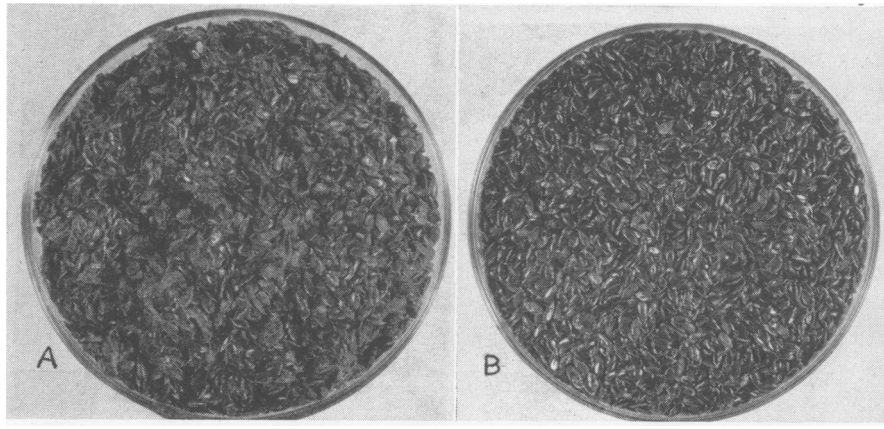

FIG. 6. Untreated flaxseed (A) and flaxseed treated with ethylene chlorhydrin at a concentration of 0.64% (B) after being stored for eight days in calorimeters.

and the treated flaxseed may be obtained from the photographs of the seed shown in figure 6. Figure 6 (A), representing the untreated control described in table III, shows that the seeds were principally dull, lumped, and for the most part, covered with a web-like growth which is the result of a heavy mold proliferation. These seeds were hot and damp and had a strong musty odor. Figure 6 (B) represents a sample of the seed treated with ethylene chlorhydrin in a concentration of 0.64%. The contrast is striking; these seeds were shiny, free-running, and not covered with mold. They were cool and dry, and smelled of fresh flaxseed when removed from the calorimeter.

VINYL PROPIONATE.—The activity of vinyl propionate was compared to that of ethylene chlorhydrin in an experiment in which 600-gram portions of flaxseed (No. 173) conditioned to a 21% moisture content were treated with this compound in quantities ranging from 0.5 to 1.5 ml. Another 600-gm. portion of the same sample was treated with 1.5 ml. of ethylene chlorhydrin. The heating curves for these samples are compared in figure

7. Vinyl propionate in concentrations of 0.29 and 0.23% on the basis of the dry weight of the seed, inhibited heating to a degree comparable to that produced by treatment with ethylene chlorhydrin in a concentration of 0.38%.

The effect of vinyl propionate upon lipolysis is evident from the data in table IV. Inasmuch as treatment with a concentration of vinyl propionate as low as 0.20% inhibits lipolysis to the same degree as does treatment with 0.38% ethylene chlorhydrin, it may be concluded that vinyl propionate is

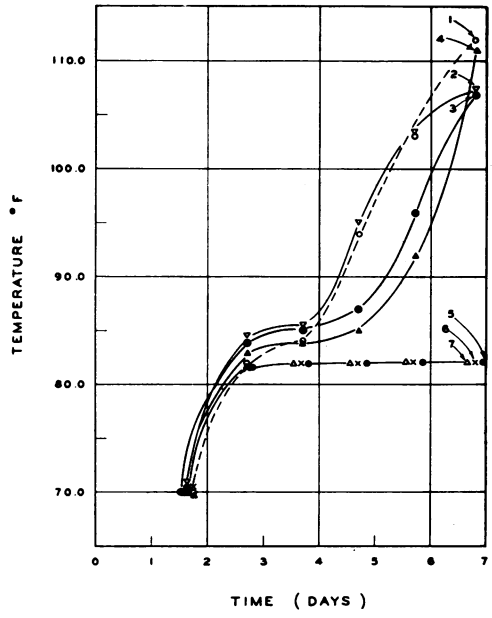

FIG. 7. Inhibition in heating produced by treatment with varying concentrations of vinyl propionate, compared to the standard treated control and the untreated control.

Curve no.	Conc. of inhibitor %
1 (untreated control)	none
2	0.09
3	0.13
4	0.20
5	0.23
6	0.29
7 ethylene chlorhydrin standard	0.38

a better inhibitor of lipolysis in flaxseed than ethylene chlorhydrin. It is interesting to note the different relationships between heating and lipolysis resulting from the treatment of flaxseed with these two inhibitors. Whereas treatment with ethylene chlorhydrin in a 0.13% concentration resulted in stimulation of lipolysis despite an appreciable inhibition of heating (fig. 5 and table III), treatment with even the lowest concentration of vinyl propionate (0.09%) inhibited lipolysis even though there was no appreciable

inhibition of heating. These results are similar to those obtained by ALTSCHUL *et al.* (5) who measured the effect of various chemical treatments on the respiration and lipolysis in cottonseed and found that the latter could be inhibited under conditions in which no inhibition of respiration occurred.

INHIBITOR INDEX.—It is quite possible for major differences in biological activity to occur between varieties of the same type of seed. In addition, conditions of growth, maturity, and harvesting may affect the subsequent behavior of different samples of the same variety of seed. These variables become still more pronounced when the seeds are conditioned to a high moisture content. It cannot, therefore, be expected that every sample of artificially conditioned seed will exhibit the same pattern of heating or the same

TABLE IV

EXTENT OF LIPOLYSIS IN FLAXSEED TREATED WITH VINYL PROPIONATE AND STORED IN CALORIMETERS FOR SIX DAYS

VOL. OF INHIBITOR	CONC.* OF INHIBITOR	FREE FATTY ACIDS†	MOISTURE
ml.	%	%	%
.....	.....	5.2	20.8
1.5	0.29	1.0	20.8
1.2	0.23	1.1	20.8
1.0	0.20	1.1	20.8
0.7	0.13	1.3	20.9
0.5	0.09	3.3	20.9
Refrigerated control‡		1.0	20.6
Ethylene chlorhydrin control§		0.9	20.8

* Per dry weight of seeds.
† Initial free fatty acid content is 1.0%.
‡ Flaxseed conditioned at a moisture level of 22% and kept at 36° F. for the experimental period of six days.
§ At the standard concentration of 0.38% based on the dry weight of seeds.

response to treatment with any given inhibitor under the above-described conditions of storage.

In order to eliminate such variables and provide a basis for comparison and a relative measure of the efficacy of any inhibitor, the inhibition in heating and lipolysis produced by treatment with 0.38% ethylene chlorhydrin (based on the dry weight of flaxseed) has been adopted as a standard of reference. In actual practice, the procedure illustrated in the preceding section with vinyl propionate has been followed. The effect of several dilutions of the inhibitor being investigated is compared with that of the standard concentration of ethylene chlorhydrin on the same lot of conditioned seed. If the standard concentration of ethylene chlorhydrin is taken as 1, then the relative efficiency of any other compound being investigated can be calculated by dividing the concentration of ethylene chlorhydrin (0.38%) by the minimum concentration of inhibitor which produces comparable inhibition. Each type of biological inhibition must be rated inde-

pendently. From the data in figure 7, the index 1.7, with respect to inhibition of heating is obtained for vinyl propionate. An index of 1.9 with respect to inhibition of lipolysis is obtained by a simlar calculation from the data in table IV. This procedure has been applied in rating the compounds previously reported to be active (**10**).

APPLICATION TO OTHER SEEDS.—Difficulties during handling and storage are common to many classes of seeds and grains. It is necessary to determine whether chemicals producing inhibition in biological activity in flaxseed by the above-described method will have the same effect when used with other classes of seeds. Some observations were, therefore, made with cottonseed, rice, and grain sorghum using ethylene chlorhydrin as an inhibitor. Samples of these seeds were conditioned to high moisture contents and

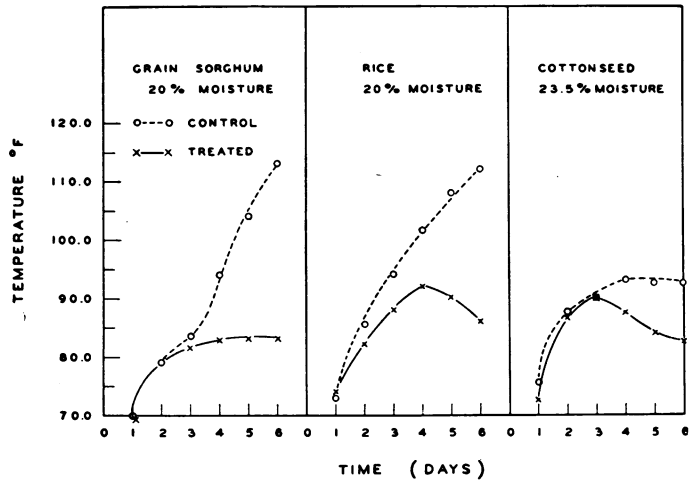

FIG. 8. The effect of ethylene chlorhydrin at a concentration of 0.38% (by weight on a dry seed basis) on the heating of high moisture grain sorghum (#185), rice (Blue Bonnett # 182), and cottonseed (Delfos #165).

portions were treated with ethylene chlorhydrin in the standard concentration and stored under the conditions described for flaxseed. Figure 8 shows the heating curves obtained for the treated and untreated samples. Under the experimental conditions ethylene chlorhydrin inhibits heating of grain sorghum, rice, and cottonseed of high moisture content in a manner similar to flaxseed. Analysis of the oil extracted from the treated and control lots of cottonseed after storage for 11 days showed that the control seed developed 6.1% free fatty acids compared to 1.3% developed in the treated seed. The fact that similar results have been obtained with ethylene chlorhydrin on four different types of seeds grown in widely separated localities and probably in the presence of a diversity of microbial populations would appear to indicate that such infections do not influence the final results under the above-described experimental conditions.

Summary

A rapid method for the measurement of the inhibition of biological activity in intact seeds has been described and applied in investigating the effects of ethylene chlorhydrin and vinyl propionate on heating and lipolysis in flaxseed. A reference standard has been established which makes it possible to assign an inhibitor index to any chemical investigated. The utility of the index has been demonstrated in the case of vinyl propionate. Fundamental and practical aspects of the application of the method are discussed.

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LITERATURE CITED

1. Altschul, A. M., Karon, M. L., Kyame, Lillian, and Caravella, Maizie. The storage of cottonseed. II. The effect of ammonia treatment on the free fatty acids and color of the seed oil, and on the rate and degree of heating of the seed. Oil & Soap **20**: 258–262. 1943.
2. ————. The storage of cottonseed. III. Factors affecting the storage of moist cottonseed. Oil Mill Gazetteer **49**(1): 8–9, 11, 13. 1944.
3. ————, Curet, M. E., Karon, M. L., Hall, Catherine, and Smith, B. A. The storage of cottonseed. IV. Mill-scale tests on the effects of various treatment of cottonseed and flaxseed on heating and free fatty acid formation during storage. Oil Mill Gazetteer **50**(8): 9–13, 23. 1946.
4. ————. A multiple point repeating switch for use with a recording potentiometer. Review of Scientific Instruments, March 1947.
5. ————, Karon, M. L., Kyame, Lillian, and Hall, Catherine M. Effect of inhibitors on the respiration and storage of cottonseed. Plant Physiol. **21**: 573–587. 1946.
6. American Oil Chemists' Society. The official and tentative methods. Chicago. 1946.

7. CLAYPOOL, L. L. and KEEFER, R. M. A colorimetric method for CO_2 determination in respiration studies. Proc. Amer. Soc. Hort. Sci. **40**: 177–186. 1942.
8. COLEMAN, D. A., and ROTHGEB, B. E. Heat-damaged wheat. U. S. Dept. Agr. Tech. Bull. 6. 1927.
9. ————, ————, and FELLOWS, H. C. Respiration of sorghum grains. U. S. Dept. Agr. Tech. Bull. 100. 1928.
10. CONDON, MARJORIE Z., ANDREWS, F. R., LAMBOU, MADELINE G., and ALTSCHUL, A. M. The inhibition of heating and lipolysis in seeds. Science (In press). 1947.
11. KARON, M. L., and ALTSCHUL, A. M. Effect of moisture and of treatments with acid and alkali on rate of formation of free fatty acids in stored cottonseed. Plant Physiol. **19**: 310–325. 1944.
12. LARMOUR, R. K., CLAYTON, J. S., and WRENSHALL, C. L. A study of the respiration and heating of damp wheat. Canadian Jour. Res. **12**: 627–645. 1935.
13. MALOWAN, J. Some observations on the heating of cottonseed. Cotton Oil Press **4**: 47–49. 1921.
14. ————. Changes in cottonseed during heat and storage. Cotton Oil Press **5**: 40–43. 1921.
15. MILNER, MAX, and GEDDES, W. F. Grain storage studies. II. The effect of aeration, temperature, and time on the respiration of soybeans containing excessive moisture. Cereal Chemistry **22**: 484–501. 1945.
16. ————, and ————. Grain storage studies. III. The relation between moisture content, mold growth, and respiration of soybeans. Cereal Chemistry **22**: 225–247. 1946.
17. ————, and ————. Grain storage studies. I. Influence of localized heating of soybeans on interseed air movements. Cereal Chemistry **22**: 477–483. 1945.
18. RAMSTAD, P. E., and GEDDES, W. F. The respiration and storage behavior of soybeans. Minnesota Agr. Exp. Sta. Tech. Bull. 156. 1942.
19. SALLANS, H. R., SINCLAIR, G. D., and LARMOUR, R. K. The spontaneous heating of flaxseed and sunflower seed stored under adiabatic conditions. Canadian Jour. Res. **22**: 181–190. 1941.