Y = per cent of dry lint in the "hull fibre."

$$58.7\,X + 6.6\,Y = 100\ (L—M)$$

$$X + Y = 100—M$$

$$\text{Per cent hull} = X\left(\frac{100}{100\text{-}M}\right)$$

$$\text{Per cent lint} = Y\left(\frac{100}{100\text{-}M}\right)$$

Example: A sample of "hull fibre" which contained 6.1 per cent of moisture lost 26.2 per cent of its weight when subjected to the above-described alkaline treatment.

$$58.7\,X + 6.6\,Y = 2010 = 100\ (L — M)$$

$$X + Y = 93.9 = (100 — M)$$

X = 26.7 per cent of dry hull

Y = 67.2 per cent of dry lint

$$\text{Per cent hull} = 26.7\left(\frac{100}{93.9}\right) = 28.4$$

$$\text{Per cent lint} = 67.2\left(\frac{100}{93.9}\right) = 71.6$$

THE OXIDATION OF FATS IN STORAGE

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THE rate of oxidation of fats in storage is influenced by the following factors: (1) The kind and amount of unsaturated fatty acid constitutents of the glycerides; (2) those constituents which act as antioxidants and prooxidants; (3) the conditions to which the fats are subjected.

In the present discussion an attempt will be made to show the relative effect of each of these factors on determining the susceptibility of various fats to oxidation.

The term "tallowiness" will be employed to denote those flavors and odors which are the result of oxidation while the term "rancid" will be used to denote other flavors and odors except "fishiness," which results from the normal hydrolysis of a fat.

Normal Constituents of Fats

Free fatty acids—Of the normal constituents affecting oxidation the amount of free fatty acids is the most easily controlled. It has been known for many years that fatty acids are prooxidants. Harris (1) finds that the wet method of rendering fats causes a high free fatty acid content. He also observes that lard of low free fatty acid remains sweet even when kept in storage for a long time. Rogers and Gray (2) observed that butter made from sweet cream kept better in storage than butter made from sour cream. This difference in keeping quality is probably due to the lower free fatty acid content of the sweet cream butterfat. Butter made by this method may be kept in storage for months without much deterioration.

To determine the effect of fatty acids on oxidation, comparable quantities of different acids were added to 10 cc. samples of fresh butterfat. The samples were exposed to air in the absence of light. As soon as one of the samples had lost its color, all samples were removed and the extent of the oxidation was determined. Table I shows the progress of the oxidation.

Unless otherwise stated the fat employed in this discussion is butter fat.

TABLE I.—CATALYTIC EFFECT OF FATTY ACIDS

Sample	Color	Relative extent of oxidation*
Control	+ + +	0.0
" + butyric acid	+ +	1.0
" + caproic acid	+	1.2
" + oleic acid	— (bleached)	12.0

*Determined by Kreis test (10).

In view of this catalytic action of the fatty acids in the oxidation of fats an attempt was made to improve the keeping quality through a reduction of the free fatty acid content by neutralization and washing.

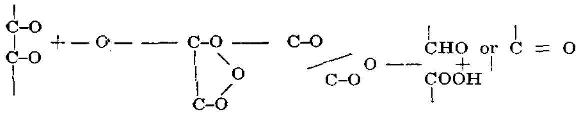
Dilute NaOH was first employed, using phenolpthalein as an indicator. After neutralization, the butterfat was thoroughly washed and dried, and found to be slightly acid. This acidity was probably due to further hydrolysis of the glycerides. This fat resisted oxidation for only one hour at 95° C., while the control resisted oxidation four hours at the same temperature.

Neutralization with $Ba(OH)_2$ and $Ca(OH)_2$ was employed, but the results were similar. Total or partial removal of the free fatty acids from a fat through treatment with alkalies and washing seemed to lower the keeping quality. This explains to some extent the loss of keeping quality in alkali refining of cottonseed oil.

In an attempt to isolate the antioxidants in cottonseed it was found that the only extract having any antioxygenic value was water soluble and acidic. Therefore, in alkali refining this substance would be lost.

An attempt was then made to remove some of the acids through steam distillation. Accordingly, 400 cc. of butterfat were treated by passing steam through the fat. At intervals of 15 minutes, samples were taken and the acidity determined. As soon as two consecutive samples gave the same acidity the treatment was discontinued. Continued treatment would result in hydrolysis of the glycerides.

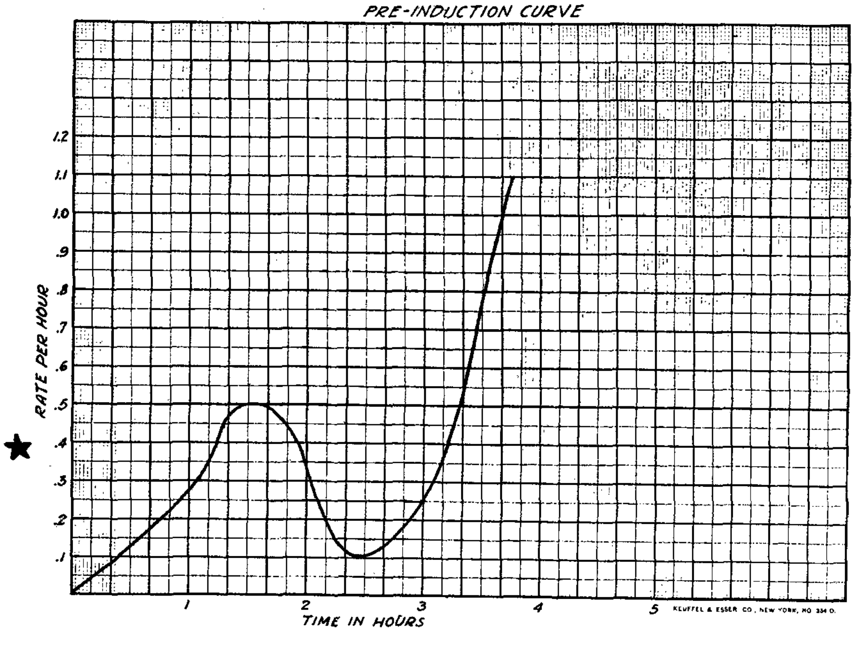
A number of samples prepared in this manner were dried and sealed under high vacuum (0.1 mm. Hg). One of these samples after exposure to direct sunlight for three months, and subsequent storage in diffuse light of the laboratory for three years, showed a negative Kreis test. The untreated samples, stored under similar conditions were completely bleached and tallowy in three weeks. The free oxygen necessary to produce such a change could not have been present in the latter under the conditions. A source of oxygen must, therefore, be assumed (5). Oxidation in vacuum may be explained by assuming the existence of loosely bound oxygen compounds. These labile compounds probably break down in storage liberating free oxygen which attacks the oleic acids radicle at the double bond. This loosely bound oxygen may be the source of the active oxygen which Tschirch and Barben (6) assumed in their theory of oxidation. The following schematic formula shows the action of active oxygen:



The active oxygen unites with the peroxide to form the ozonide which is very unstable and breaks up to form one molecule of an aldehyde, or the corresponding ketone, and a molecule of an acid.

The reported experiment substantiates the claim of various writers that the presence of air is not necessary for the oxidation of fats. Possibly the oxygen was derived from losely bound oxygen compounds. Whether the steam caused the active compound to break down or be volatilized is not known. Evidence to be presented may indicate either action.

During the oxidation of large quanties of butterfat there were irregularities in the rate of absorption during the induction period. Closer study of this absorption showed that the rate passed through a minimum just prior to the period of rapid oxygen absorption. The nature of this reaction is shown in Figure 1.



*Rate of Oxygen Absorption within the Induction Period.

FIGURE I.

This would indicate that some minor constituent is being oxidized, otherwise the absorption would continue. This may be due to the oxidation of the loosely bound compounds to a more stable form, or to a form that promotes the subsequent rapid absorption.

The volatility of prooxidants may be shown in a very striking manner, as follows: Connect in series three small vessels containing equal amounts of fresh dry butterfat in such a manner that the same air may be blown successively through the three samples. Immerse the three samples in a water bath held at 100° C. and pass air through them. A series of samples treated in this manner showed oxidation as indicated by bleaching and Kreis test in the various times noted: No. 1, 16 hrs.; No. 2, 13 hrs.; and No. 3, 6 hrs. The numbers of the samples indicate the order in which air passed through the vessels. It is evident that prooxidants are removed from No. 1 and increased in No. 3.

Pigments.—Certain oils, especially those of plant origin, contain carotenoid pigments. These pigments are also constituents of some animal fats, especially butterfat. That these pigments influence the rate of oxidation has been shown by Olovitch and Mattill (7) who maintain that carotene is a prooxidant. Monahan and Schmitt (8), as well as Richardson and Newton (9), classify this compound as an antioxidant.

Since the effect of the natural pigments on keeping quality has been controversial, a study was made of their action. The effect of lipochromes, carotene and lycopene; and a flavone, quercitin, was studied by adding the pigments to different samples of the same fat. The lipochromes acted as prooxidants while quercitin acted as an antioxidant.

Recent work in this laboratory has shown that certain substances may be either antioxidant or prooxidant, depending upon their oxidation-reduction potential and dissociation. A better knowledge of antioxidants and their modes of action may explain some of the apparent contradictions.

Processing and storage conditions.—Air, heat, light and moisture have been mentioned as accelerators in the oxidation of fats. Authorities generally agree that air, light and heat accelerate the reaction, but there are conflicting views regarding the relative importance of each. This can be attributed largely to the lack of methods for detecting and following changes due to oxidation.

In recent years considerable progress has been made along this line (11), (12), (13) and by utilizing these methods an attempt has been made to study each factor separately.

Since most of these factors cannot be entirely eliminated, it seems necessary to describe rather completely the experimental conditions. The effect of air is considered eliminated when a fat is sealed in a vessel under a pressure of less than 0.1 mm. of mercury. Repeated evacuation and refilling with purified nitrogen were employed to reduce the possibility of any dissolved oxygen remaining in the fat.

A fat was considered to be dry after it was centrifuged and held at 60° C. for 1 hour, at a pressure of less than 0.1 mm. of mercury. During the drying there was constant agitation.

The action of heat is shown by the changes produced by different temperatures when the samples were held in the dark and in the absence of air and moisture. The action of light is considered to be the only factor, when dry fats are sealed under an air pressure of less than 0.1 mm. of mercury and immersed in a brine held at —10° C.

Air and heat.—Holm (17) has found that the rate of autoxidation of fat is very slow at temperatures below —10° C., therefore these studies will be limited to temperatures of 0° C. and above. The effect of air is shown by comparing the changes that occur in air (not circulating) and in a vacuum in the absence of light. Table II shows the effect of air and heat as indicated by change in the induction period (11).

TABLE II.—THE EFFECT OF AIR AND HEAT UPON OXIDATION OF FATS IN THE ABSENCE OF LIGHT

		0.1 mm. pressure Hg.		760 mm. pressure Hg.	
Time of storage (Months)	Temperature (° C.)	Induction period (Minutes)	Per cent of original induction period (Per Cent)	Induction period (Minutes)	Per cent of original induction period (Per Cent)
0	0	248	100	248	100
1	"	230	93	210	85
2	"	209	83	187	75
3	"	200	80	160	64
0	22	248	100	248	100
1	"	200	80	173	70
2	"	180	73	131	53
3	"	103	42	67	27
0	32	248	100	248	100
1	"	171	69	110	46
2	"	113	46	48	19
3	"	52	21	43	17
0	42	248	100	248	100
1	"	154	61	92	39
2	"	75	30	43	17
3	"	41	17	47	18

Table II and the theory of loosely bound oxygen compounds may be used to explain the deterioration of fats in vacuum. Experience has shown that storage in vacuum or in inert gases may retard oxidation, but not prevent it.

Light.—To eliminate uncontrolled variation in the action of the other factors all samples were dried, sealed in vacuum, and held at —10° C. The results obtained in this study are given in Table III.

TABLE III.—THE EFFECT OF LIGHT UPON THE OXIDATION OF FATS.*

Time of exposure in Months	Relative keeping quality when exposed at .1 mm. pressure Hg. Light	Dark	Relative keeping quality when exposed at 760 mm. pressure Hg. Light	Dark
0.....	100	100	100	100
1.....	80	98	67	95
2.....	72	93	50	93
3.....	69	88	39	84
4.....	62	..	36	..

*Diffuse light.

The accelerating action of light seems to be much more pronounced in the presence of air.

To determine the nature of the oxidation in light, purified dry air was passed through cottonseed oil. The extent of oxidation was followed by measuring the increase in weight of the oil and by the H_2O and CO_2 formed during the experiment. In the dark there was found to be an increase in weight, and H_2O was formed but no CO_2, as shown in Figs. II and III. In the presence of light the increase in weight of the oil was greater, more water was formed and in addition appreciable amounts of CO_2 were formed. Figure IV shows the relative amount of CO_2 formed in light and in the dark.

It is evident that light causes a greater degree of oxidation. Figure V shows the action of light on the oxidation of fat in the absence of free oxygen. In this experiment purified dry nitrogen was passed through the fat. The loss in weight and the H_2O produced were determined. There was no CO_2 produced. The effect of light shown in Figure V is presumably the splitting off of loosely bound oxygen.

The marked effect of light in the presence of air suggested a study of the action of intense light on the oxidation of fat. A sample of fat exposed to an intense beam of light was shaken and the oxygen absorption measured. In the course of 7 hours about 8 cc. of oxygen were absorbed. The same system standing overnight absorbed a similar volume of oxygen (14). This continued absorption after irradiation is not observed when oxidations are accelerated by heat.

Recent work has shown that the red end of the visible spectrum is most detrimental to the preservation of fats (14). In view of this fact, any medium which absorbs light of the red end of the spectrum should offer protection. The narrower the transmission band the greater will be the protection offered. Certain colored glasses or films which have been patented owe their protection to the exclusion of the major portion of the light or to lowering intensity rather than to limiting the band of transmission.

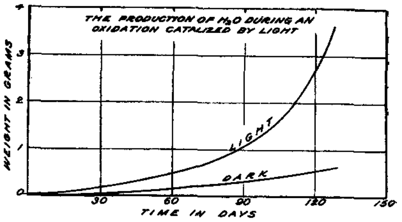


FIGURE III.

Moisture.—Holm (15) has found that moisture may be either an antioxidant or prooxidant, depending on the percentage present. He found that an extremely low moisture content was conducive to the development of "tallowiness," while a slightly higher moisture content prevents the development of this off-flavor. He postulated, that when the moisture is very low the oxidation stops at the aldehyde stage, giving rise to objectionable flavors; and when the moisture is slightly higher, the oxidation proceeds to the acid stage. The milk powder and cereal industries have employed this principle in the control of their products

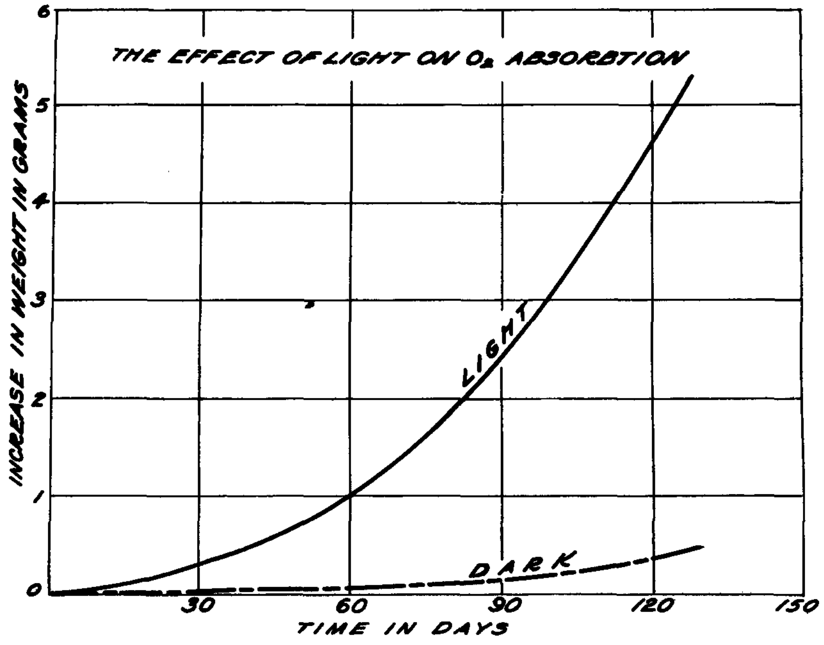


FIGURE II.

with a greatly diminished loss due to off-flavors.

In order to determine the effect of moisture on keeping quality, a fat was shaken with water and lightly centrifuged to remove water globules. In this manner a fat containing about 0.15% moisture was obtained. These samples were stored at 0° C. in air and under vacuum. Data summarizing these results are given in Table IV.

TABLE IV. THE EFFECT OF MOISTURE ON KEEPING QUALITY OF FATS IN STORAGE

Time of storage Months	Tempera-ture °C.	Relative change of keeping quality at 0.1 mm. pressure Per cent *		Relative change of keeping quality at 760 mm. pressure Per cent *	
		Moist	Dry	Moist	Dry
0	0	100	100	100	100
1	"	89	93	78	85
2	"	76	83	71	75
3	"	62	80	51	64

*Per cent of original keeping quality.

Newton (16) has found that moisture destroys the activity of antioxidants. Recent work in these laboratories has shown that the same substance may be either an antioxidant or prooxidant, depending on the state of dissociation. Moisture, without doubt, causes dissociation of the antioxidants. Maleic acid, a powerful antioxidant in dry fat, is without value as an antioxidant where much moisture is present.

The effect of air, light, heat, and moisture, have been studied. In each case an attempt has been made to eliminate the other factors. In the storage of fats more than one of these factors are usually present. A study was then made to determine the combined action of some of them. Since storage is either in the presence or absence of air, a study was made both in air and vacuum. Table V shows the effect of heat, light, and moisture in the presence of air.

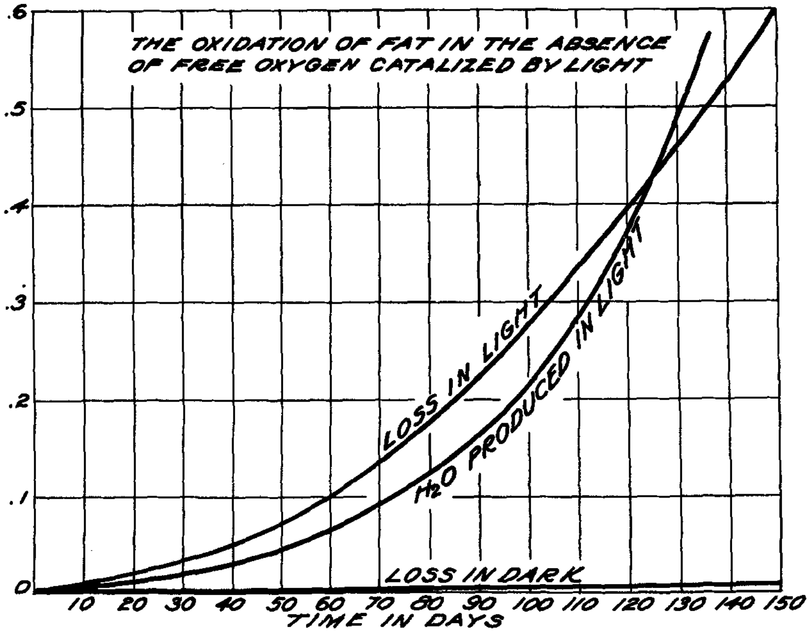


FIGURE IV.

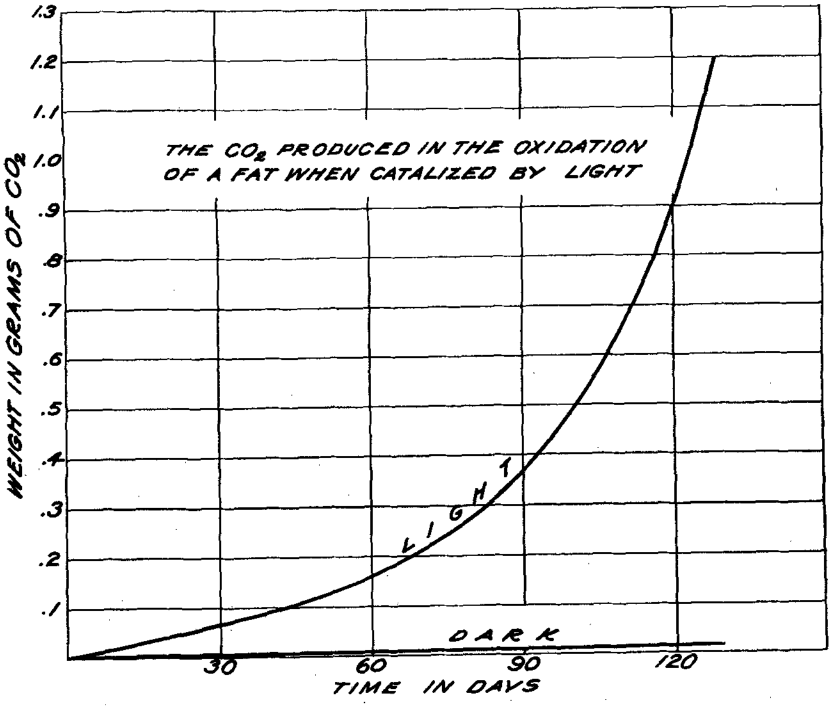


FIGURE V.

TABLE V.—THE EFFECT OF HEAT, LIGHT, AND MOISTURE IN THE PRESENCE OF AIR.

Accelerator	Relative change in keeping quality in 3 months
Control*	100%
Heat + air (0°C.)	36% decrease
Diffuse light + air (—10°C.)	64% "
Moisture (0.15%) + air (—0°C.)	49% "

*At time of storage.

TABLE VI.—THE EFFECT OF HEAT, MOISTURE AND LIGHT IN THE STORAGE OF FATS IN THE ABSENCE OF AIR.

Accelerator	Decrease in keeping quality. 3 months at 0°C. and 0.1 mm. pressure of Hg.
Control	100%
Heat	20% decrease
Diffuse light + heat	35% "
Moisture 0.15% + heat	38% "

In the presence of air, light is the most powerful accelerator, and moisture and heat follow in the order given. This conclusion holds only for the lower temperatures. Again it is evident that a vacuum may retard oxidation, but not prevent it.

An attempt has been made to show the relationships between storage conditions and the oxidation of fats. Present-day knowledge of the oxidation of fats leaves much to be desired in such a study. This lack of fundamental knowledge is responsible for the lack of better methods of study. It is hoped that this paper will stimulate a study of the fundamental basis of the oxidation of fats.

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REPORT OF THE SEED ANALYSIS COMMITTEE*

THE activities of the Seed Analysis Committee this season have developed very few positive results on which recommendations may be made. However, certain lines of investigation were followed and will be briefly reported on.

The efforts of the committee to find or develop a mill more suitable for grinding seed samples were continued without success. Manufacturers in all parts of the country were contacted without finding a satisfactory unit or interesting anyone in developing one. E. H. Sargent & Company also unsuccessfully tried to get some manufacturer to cooperate with them in this work. The explanation seems to be that the market would be so limited that development of a grinder would not be profitable to the manufacturer. The committee still feels, however, that the present grinding operation is weak and presents a large opportunity for the introduction of variables and error, even with the most careful attention.

A method for the determination of lint on cotton seed, developed by Mr. R. S. McKinney of the Oil, Fat and Wax Laboratory at Washington and submitted to the committee at their request, was also studied. Without question, more and more frequently the chemist (and especially the plant chemist) is asked to determine the percentage of lint on seed, both before and after mechanical delinting, usually as a function of plant control. For this type of work the method is entirely adequate. However, the practical difficulties in obtaining a sample, small enough for use representative of the whole, are such that the committee feels a great deal of confusion might develop if any such test later became accepted as a settlement test. It therefore confines its recommendation to the statement that the method is simple and gives results that may be closely duplicated using any given sample. The question of the desirability of including it among our Official Methods we feel should be decided by the membership.

The third phase of the committee's work centered about the questionnaire which was sent to the check seed collaborators. Before discussing this phase the committee and its chairman would like to thank the membership for their cooperation and interest as shown by the completeness of the replies and suggestions on the questionnaire. In spite of this, the committee's study of these replies in conjunction with the check seed series results, did not equal our hopes. No absolutely undebatable trends or facts were brought out; it was hoped that common differences in procedure might be found in similar groups, thus showing definite effects produced from specific causes. This did not prove entirely true. Laboratories whose questionnaires showed almost identical procedures were found at opposite extremes in efficiency, while no single variation in temperatures or procedure could be found solely in one group. Certain tendencies, often somewhat obscured by other factors and of a negative rather than a positive nature, did seem visible; whether or not these tendencies are the primary causes of error might be debatable. However, it is significant that no laboratory incorporating any one of the following points in its procedure rated high in accuracy:

No laboratory using a maximum fuming temperature as high as 135° C. missed less than eight tests.

Laboratories using unmeasured or greater amounts than 1.5 cc. of acid tended definitely to fall in lower efficiency groups, with the exception of instances where extremely low fuming temperatures were used.

Laboratories designating their ground samples as brownish or dark (with one exception) missed nine or more tests.

Ground samples designated as gray or yellowish are, from the report, equally satisfactory.

From the above it is obvious that carelessness or a deliberate disregard of the rules is one of the major causes of error. Too high fuming temperatures and varying amounts of acid have no other explanation. Darkened samples, while more difficult, can in all cases be eliminated by careful experimentation, except where the seed itself is so badly off as to have a naturally brownish color.

One other point seems worthy of mention: Laboratories using lower than required temperatures for fuming did so with no apparent damage to their efficiency. One laboratory using an extreme fuming temperature of from 60° to 80° C. had only five tests outside of tolerance. The width, thus shown, of the effective fuming range below that now stipulated and the narrowness of the range from it to temperatures high enough to be harmful (135° C.) logically raise a question regarding the present rule. It would seem possible that, instead of being the optimum, the present designated fuming temperature may lie too near the maximum limit, thus requiring very close control and offering the possibility of overheat-

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*As presented at the Spring Meeting at New Orleans, May 28-29, 1935.