of these compounds was investigated, and the optimum conditions were determined. Measurements of the surface-active properties, including pour foam, soil removal, and redeposition, of several of the acylated derivatives are described, with reference to Igepon T.

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Rancidity as a Factor in the Loss of Viability of Pine and Other Seeds

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THREE YEARS AGO the Southern Forest Experiment Station of the United States Forest Service and our laboratory started experiments on the preservation of pine seeds. Pine reforestation has become an important project of the U.S.D.A. Pine trees under Louisiana's conditions produce a normal crop of seeds at irregular intervals; the necessity therefore of storing the seeds of high yield years in order to meet the needs for seeds during the light crop years is apparent. Our experiments in this field deal not only with the preservation of seeds at various temperatures, dehydrofreezing techniques, and storage under an inert gas but also extend to a more fundamental study of the viability process in general, the development of rapid color methods for measuring viability using triphenyl tetrazolium chloride, and the development of treatments which might improve germinability. We thought also that since pine seeds are relatively rich in oil, the rancidity of the fat of the seeds might have something to do with the loss of viability.

The effect of rancidity of the fat on the vitamins and on other oxidizable compounds of foods is a well established fact (17). That vitamins together with other substances are important factors in conditioning germination of pollen (3), rice (16), and other seeds has also been shown by various experiments. To suppose therefore that a connection might exist in seeds rich in fat between its rancidity and the germinability of the seeds is quite a logical hypothesis. To ascertain however such a relationship on a strict cause-and-effect basis, if the hypothesis is correct, is not quite such a simple matter, considering our present limited knowledge concerning the various phases of rancidity development and the uncertainty of methods for measuring it (10). Our experiments on the study of germination of various seeds, *viz.*, rancidity development in the fat, indicate that a connection between the two does exist; but because of the complexity of factors determining viability and the variability of the individual seeds it is rather difficult to establish a strict correlation between the two in a preliminary experiment with a limited number of tests.

The data of Tables I and II show that seeds of high germinability have no actual rancidity in their fat and as the viability decreases, so the rancidity (peroxide value) increases more or less steadily. This correlation between viability as determined by sand flat germination tests and color tests does not hold well, especially in the low range of viability values. It seems rather certain that rancidity development is one among several other factors contributing to the loss of viability, such as protein coagulation, degeneration of oxidizing and digestive enzymes, exhaustion of stored foods, cell poisoning, etc. (2), which under certain conditions of storage undergo changes independently of rancidity development. Such might

TABLE 1
Rancidity and Other Fat Changes During the Gradual Loss of Germinability in Longleaf Pine Seeds

Storage conditions	Moisture	Fat (dry basis)	Germination (sand flat germ. test)	Free fatty acids	Peroxide value	Iodine number (Hanus)
	%	%	%	%	*mM/Kg.*[a]	
Fresh seeds (partially dried in air)	14.49	25.09	80	0.28	0.0	—
Fresh seeds (partially dried in air)	10.90	25.43	75	—	0.0	147.0
Seeds stored at 25°F. in burlap bag for 2 years	12.93	23.43	70	2.31	9.5	146.6
Seeds stored at 45°F. for 1 year in jars	13.96	23.08	48	4.51	25.0	—
Seeds stored in jars at room temp. for 1 year	6.53	23.72	18	2.82	70.0	—
Seeds stored in jars at room temp. for 2 years	7.96	24.50	0.0	2.54	130.0	141.7
Seeds stored in jars for 2 yrs. at 45°F	14.60	23.96	0.0	2.61	—	143.8
Seeds stored at 45°F. in CO_2 + ethylene oxide for 1 year	13.86	24.90	0.0	2.68	105.0	—
Seeds stored in CO_2 + ethylene oxide at room temp. for 1 year	11.29	24.60	5.0	1.97	55.0	—

[a] mM = millimoles.

TABLE II

Germination Tests *viz.* Rancidity of the Fat in Okra Seeds

Variety and time of storage at 35°F. in bags	Moisture	Fat (dry basis)	Free fatty acids	Peroxide value	Viability	
					Sand flat germ. test	Color test
	%	%	%	*mM/Kg.*	%	%
Var. La. Market stored 4½ years	9.25	18.12	14.33	22.5	0.0	0.0
Var. La. Market stored 2½ years	11.30	18.08	13.30	14.0	58.0[a]	80.0
Var. La. Market stored 1½ years	6.73	16.38	11.26	11.5	90.0	85.0
Var. Green Velvet stored ½ year	5.70	18.20	0.77	2.5	95.0	92.0

[a] Plus some 25% underground germination (the seeds showed signs of germination, but they did not develop viable seedlings).

be the case, for instance, in the samples of Table I, which were preserved by gas storage in carbon dioxide containing some ethylene oxide (11). The samples of Tables I and II have different moisture contents which affect both rancidity and viability somewhat differently, but since our experiment was planned primarily for the preservation of viability, we had to adjust the moisture content of the seeds to the best conditions for the preservation of viability; at room temperature, for instance, viability of pine seeds would not last long if their moisture content were not reduced to a sufficiently low level.

The fact that rancidity has something to do with the loss of viability of the seeds suggests the possibility of other treatments of the seeds, such as coating, use of antioxidants or even storage under an inert gas at a low level concentration which might protect the seeds from rancidity development and at the same time would not adversely affect their viability; such treatments look very promising, especially in storage at room temperatures where the loss of viability is very quick, for many kinds of seeds.

When these experiments on the subject of rancidity, *viz.* germinability, were practically ended, a work of Mirov on the possible relation of linolenic acid to the longevity and germination of pine seeds came to our attention (18). This paper deals with storage experiments on two kinds of pine seeds: the *Pinus Jeffreyi,* the seeds of which can be stored in closed containers at room temperature for a long time without loss of their viability, and the sugar pine seeds *(Pinus lambertiana),* which under the same conditions quickly lose their viability. The first seeds have an iodine number of their fat of 136.4 whereas the second ones have 150.0.

Because of the iodine number changes of the respective fats in the two kinds of seeds, the author suggests that the linolenic acid, which at room temperature disappears more rapidly during the storage of sugar pine seeds than of Jeffreyi seeds, might play a role in the germination and the longevity of oleaginous seeds. As is well known, the higher rate of disappearance of linolenic acid actually means a higher rate of rancidity development; and between two equally probable explanations the simplest and more general must prevail. Which of the two explanations is more simple, more general, and more easily demonstrable by the evidence of existing facts, will be seen from the following discussion. The linolenic acid hypothesis is based on two rather improbable assumptions: a) the assumption that the respiration of fatty seeds depends solely on linolenic acid, which has to be proven before it is acceptable; b) the indirect evidence for the role of linolenic acid in germination based on the iodine number changes which we know are only appreciable in a rather advanced stage of fat deterioration whereas the viability of seeds is probably lost much earlier and before such a stage is actually reached (12). In our opinion Mirov's hypothesis needs further evidence and support from respiration studies and direct linolenic acid measurements before it can be accepted as a possible explanation of the role of linolenic acid in the loss of viability of seeds.

The rancidity hypothesis, on the other hand, besides the experimental data of Tables I, II, and IV with pine and okra seeds receives wide support from the facts of experience we have in dealing with the germination of seeds in general. We know, for instance, that seeds which contain antioxidant substances like sesame seeds (13), tomato seeds (6), and aniseeds (9), preserve quite well their germinability during storage; and, in general, storage conditions which favor the development of rancidity in the fat independently of the presence of linolenic acid increase the rate of loss of germinability and *vice versa.* Among the same kind of seeds, varieties which by the special structure of the coat offer protection from the oxygen of the air and its moisture, keep their viability better during storage as, for instance, the late soya varieties of the Pelican type with the waxy coat compared with the early ones (Dortchsoy, Ogden, etc.) with the chalky and easily cracked coat.

TABLE III

Average Percentage of Loss of Vitality of Different Kinds of Seeds When Kept Under Different Conditions for 243 Days in Eight Different Stations of the U.S.A.

Kind of seeds	Trade conditions		Dry rooms		Basements	
	Envelopes	Bottles	Envelopes	Bottles	Envelopes	Bottles
Tomato	5.20	0.20	3.29	0.44	13.63	0.30
Peas	11.45	0.47	15.94	0.58	36.62	0.60
Watermelon	12.37	0.99	10.44	3.03	21.52	1.59
Lettuce	15.76	1.29	15.14	1.49	28.95	1.65
Radish	22.67	6.65	18.37	7.73	25.13	6.00
Sweet-corn	26.09	32.55	25.06	48.00	31.74	22.71
Bean	29.59	1.72	29.76	1.36	43.61	10.00
Cabbage	43.56	1.94	33.44	2.67	42.29	0.22
Carrot	54.50	1.38	34.35	8.89	53.96	9.50
Onion	74.11	1.20	37.12	4.80	65.90	6.33
Pansy	84.91	15.60	53.97	23.02	84.76	27.49
Average loss of vitality	36.63	3.92	21.19	8.08	42.28	4.51

Table III is taken from the work of Duvel on the vitality and germination of various seeds throughout the U.S.A. (5). One can see the great difference in loss of viability of seeds preserved in envelopes and in sealed bottles. This difference can hardly be explained only by the factor of moisture as the author seems to believe, especially when we consider the differences in dry-room storage. There are two other factors involved in this experiment besides the absorption of moisture by the seeds: the reduced respiration and the inhibited autoxidation of the fat because of the more or less anaerobic conditions prevailing inside the bottles as a result of the carbon dioxide formed by the respiration of the seeds. We

know already from experiments with foods in general (14) that carbon dioxide storage decreases respiration in tissues and retards rancidity development in the fat.

The fact that Kinman (15), working with sesame seeds, acquired evidence of a certain correlation between seed germination and free fatty acids may be explained by the correlation which exists to some extent between rancidity and fatty acid production. Had the author, besides the free fatty acids, determined also the rancidity in the fat, a better correlation might be obtained. Acid development and rancidity sometimes go together but not always parallel to each other. In cotton seeds for instance Altschul failed to find any correlation between free fatty acids and germinability (1). We may now offer the following suggestion for the support of the hypothesis regarding the role of rancidity in germination. The role played by tocopherols as antioxidants is well known. Tocopherols (β and δ) are widely distributed in plant seeds. Assuming that tocopherols, which have vitamin E activity and are associated with fertility and longevity in animals (7), have also a similar function in plants, then the role of rancidity in destroying germinability becomes understandable.

Another interesting and rather revealing point found in the above-mentioned important Duvel's experiments on the vitality of seeds is the fact that viability is more or less stable in the beginning of storage for a certain period of time, depending on the kind of seeds and the method of storage, and then begins to decline rather quickly. This can be interpreted as an indication of the existence of some factor or factors in the seeds (such as antioxidants like tocopherol, for instance) which by their presence prevent the deterioration of life. Once the protective factor disappears, the process of life disintegration takes its normal course, following the general law of chemical reactions with an accelerated speed as if the products of such reaction acted as a catalyst in the process. This scheme again fits well the hypothesis of tocopherol-rancidity-viability relationship. When tocopherol disappears from the scene as the antioxidant in the chain reaction of autoxidation of unsaturated fatty acids and the rancidity products (peroxides, radicals, etc.) begin to accumulate in the cells, then the essential elements which sustain the life such as vitamins, enzymes, and others, begin to undergo down-grade changes with an accelerated speed. This is always the case with processes associated with rancidity development, to the point of maximum entropy for the particular living system of seeds, which is actually the point of death. As a matter of fact, tocopherol in the living system is known to affect changes in enzymatic activity and to exert protection of vitamins and sulfur containing amino acids (8).

Although the explanation given by Mirov for the loss of viability in oleaginous seeds is different from our interpretation of his data and of ours, both explanations have this in common. We both accept the fact that fat changes can cause loss of germinability in oleaginous seeds. This by itself is an interesting result from a practical standpoint because it suggests the possibility of the use of special methods for preventing the loss of viability in seeds, which undoubtedly will result in a considerable saving of money to the farmers in general.

In order to test the possibility of such a treatment we dipped pine, okra, and onion seeds in a coating solution containing starch phosphate. Starch coating of carrots is known to prevent losses of carotene during drying and storage (19). Starch phosphate also has been reported in a patent as having antioxidant properties for oils (4). The seeds were dipped in a 1% solution of starch phosphate, obtained from the Research Department of the Corn Products Refining Company since starch phosphates are not yet available on the market. The moisture of the dipped seeds was brought back practically to its original value by a rapid treatment at room temperature with dehumidified air in a Desomatic Dehumidifier, Unit DOR 38, and the treated and untreated (control) seeds were placed in jars and stored at room temperature.

The room temperature in our laboratory during the months of storage fluctuated around 30°C. during the day, but it was somewhat lower during the night. Viability tests were made at the beginning and at various intervals during the storage as well as at the end. Moisture, fat, and rancidity tests were made at the end of the storage. The results of these tests appear in Table IV and show that the above treatment with starch phosphate was successful in preserving viability as well as in preventing rancidity development in the fat of okra and onion seeds. In view of the fact that a coating, in general, like gas storage of live seeds and fruits, in order to be successful for the preservation of life requires a delicate adjustment in regard to its effect on respiration, we consider these results rather significant for a preliminary test. We hope that in further experiments with these and other seeds the beneficial effect of coating with starch phosphate might prove more important for the preservation of viability than it appears from the results of these preliminary experiments.

Summary

A study of the factors affecting viability of pine seeds conducted for three years with the collaboration of the Washington Forest Service of Louisiana showed a certain correlation between rancidity development in the fat and the loss of their viability as tested by sand flat germination tests and color tests with tetra-

TABLE IV
Effect of Coating with Starch Phosphate on the Viability of Seeds

Kind of seeds	Before treatment	After 2½ mo. of storage		After 5 mo. of storage at room temperature						
	Viability (%)	Viability (%)		Viability (%)		Peroxides (mM/Kg.)		Fat content (dry basis)	Moisture (%)	
		C[a]	T[b]	C	T	C	T	C	C	T
Okra (La. Market Var.)	90	87.5	97.5	62.5	85.0	12.0	5.5	16.38	11.3	12.5
Onion (Red Witherfield Var.)	70	18.0	64.0	0.0	15.5	19.0	8.5	—	9.2	9.1
Pine (Longleaf Var.)	78	27.5	37.5	4.0	5.0	15.0	15.0	25.43	8.4	8.0

[a] C = Control. [b] T = Treated.

zolium chloride. Apparently there are other factors also affecting germination which act independently of rancidity development, but rancidity seems to play an important role in this process, at least under normal storage conditions and in the early stages of viability deterioration. The loss of linolenic acid through respiration, which has been suggested by Mirov as the cause of loss of viability and longevity of oleaginous seeds, seems to be a rather indirect factor because of the close association of linolenic and other unsaturated acids, losses, and the development of rancidity. Experiments with okra seeds showed a similar correlation between rancidity development and the loss of germinability. Preliminary experiments by coating okra, onion, and pine seeds with an antioxidant (starch phosphate) proved beneficial for the preservation of viability of okra and onion seeds during storage at room temperature.

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The Determination of Glycerine in Polyol Mixtures by Paper Chromatography

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RAPID AND ACCURATE METHODS for the determination of glycerine in polyhydric alcohol mixtures, containing polyols such as ethylene glycol, propylene glycol, erythritol, butanetriols, sorbitol, and mannitol have not been available. Precision distillation has often been used, but this technique is exacting and time-consuming. In the case of glycols and glycerine it is difficult to separate the glycerine from the glycols by distillation. A recent paper by Rosenberger and Shoemaker (7) used an azeotropic distillation for the determination of glycerine in an ethylene glycol water mixture. Dal Nogare (1), employing a partition chromatographic technique, was able to separate C_2 to C_4 glycols on a silicic acid-Celite column. However no work was done with glycerine and higher polyhydric alcohols, which would be removed very slowly from the column. We have devised a paper chromatographic technique by which glycerine can be separated quantitatively from glycols and higher polyhydric alcohols such as sorbitol, erythritol, and butanetriols. The suggested procedure may be adapted to semi-micro scale very easily and does not require the full attention of the analyst as in a column partition chromatographic technique.

In the development work on this procedure, paper chromatography was found to be the most satisfactory method to separate glycerine from other polyhydric alcohols, using Whatman No. 1 filter paper. To determine whether a descending or ascending technique would provide an efficient separation and to determine the approximate concentration of glycerine, a qualitative chromatogram was run on the unknown samples. The developing solvents for ascending and descending chromatograms are sec-butanol-water (saturated), and n-butanol-water (saturated), respectively. The glycerine is located by spraying a parallel chromatogram with an indicating reagent. The corresponding area in the unsprayed sample chromatogram is cut out of the paper and extracted with warm water. Others (2, 3, 8) reporting on the quantitative paper chromatographic separation of sugars have found that sugars are readily extracted from the paper simply by immersing the paper in water. Since this recovery is simple and requires no special devices, the authors adopted this procedure.

After elution the glycerine is determined on an aliquot of the eluate by using the procedure of MacFadyen (4). It is well known that periodate cleavage of vicinal hydroxyls will produce formaldehyde and, in some cases, formic acid (5, 6). The periodate oxidation of glycerine produces two moles of formaldehyde and one mol of formic acid. MacFadyen showed that micro quantities of formaldehyde could be determined by reaction with chromotropic acid. Thus the chromatographic separation of glycerine from the accompanying constituents, followed by the application of the two above-mentioned reactions, should furnish a method for the determination of glycerine in a glycol, polyhydric alcohol mixture.

Experimental

APPARATUS

Paper Chromatographic Chambers. These are ascending and descending types with accompanying accessories made of glass or stainless steel.

Pipettes. These are of 6 microliter capacity and volumetric 1 ml., 2 ml., 5 ml.

Glass Atomizer.

Test Tubes. 50-ml capacity.