cantly different from that in predominantly nornicotine samples.

9. Nicotinic acid was found in all samples. The quantity found in the better grades of flue-cured tobacco seems to be much lower than in air-cured samples, which is in line with the much shorter exposure of these samples to conditions under which oxidative enzymes would be expected to be active.

10. 3-Pyridyl methyl ketone, called 3-acetyl pyridine in the previous paper (*8*), was detected in half the samples reported here but only at the lower limit of the sensitivity of the methods used. In the previous paper the R_f of this compound was reported as 0.61. Later the principal color development was found at R_f 0.88, though some color remained at 0.61. The characteristic ultraviolet absorption spectrum is now found at 0.88 and not at 0.61. New solutions of the same sample of alkaloid and another independently synthesized sample obtained from P. C. Teague (*7*) also gave the principal color and the ultraviolet absorption at R_f 0.88.

11. Though the estimated number of substances isolated which show color with cyanogen bromide and *p*-aminobenzoic acid, as given at the bottom of Table I, is large in the case of all samples, most of these substances are present in very small amounts. The numbers given are based on best judgment with respect to the identity or nonidentity of substances derived from different solvent extracts of the tobacco samples. Judgment is based on similarity of the R_f value, the characteristics of the ultraviolet absorption curve, and the colors of the spots, both in visible and ultraviolet light, after treatment with color reagents. As all these criteria and especially the absorption curve can be influenced by admixture with other substances, the number of substances present which react with cyanogen bromide and *p*-aminobenzoic acid cannot be stated categorically. Additional tables showing solvent fractions from which these substances were obtained, R_f values, ultraviolet absorption curve characteristics, and calculated quantities in each sample are contained in the mimeographed report (*6*). A careful study of these tables shows that certain of these unidentified substances appear to be identical as obtained from a number of different tobacco samples.

12. It is evident from the method of calculation that the various errors involved, especially in the assumptions used, would tend to pile up in the "Not Isolated" position of Table I. Eight to 25% of the total "alkaloidal" material falls in this class in different samples, but there is no obvious relationship between the size of this quantity and the character of the sample. Much of this material may not be 3-pyridines.

13. More detailed data and results are available as a mimeographed report (*6*).

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Received for review March 16, 1955. Accepted May 5, 1955.

GRAIN STORAGE

Effects on Corn of Storage in Airtight Bins

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ONE OF THE PROBLEMS associated with modern mechanized methods of grain harvesting is the increased amount of grain harvested at moisture contents above the safe storage level. Mechanical drying methods suitable for removing the excess moisture have been widely explored and considerable practical development work has been accomplished. As an alternative to drying, hermetic or sealed storage bins have been suggested for holding grain of high moisture, principally feed grains, on the farm. Spoilage of high moisture grain is primarily due to microorganisms. Exclusion of oxygen from the storage promises to reduce this cause of grain spoilage.

The principal development of hermetic storage for damp grain has been in France. Vayssière (*7*), reporting to the Food and Agriculture Organization of the United Nations in 1947, described a hermetic storage as one where the product is protected from any exchange of gases or liquids from the outside environment. In the storage of high moisture grain, mold and overheating were forestalled without preventing development of acidity caused by anaerobic fermentation. Other advantages claimed by French investigators were the suffocation of insects and other pests present at the time of storage and the positive prevention of the movement of insects and moisture into the storage.

Oxley (*6*), British grain storage investigator, states that the principle on which airtight storage is based is the inhibition of aerobic organisms (which include the majority of fungi) by the depletion of oxygen and production of carbon dioxide in the interseed spaces. Oxley also reports that according to British observation of work in France by Blanc and others, airtight storage was satisfactory for preserving the condition of wheat at 16.3% moisture for 18 months. Other lots stored at 18.9% appeared satisfactory, but these observations were not conclusive.

Underground storages for grain have been used in some countries for many centuries. The exchange of air in underground storages may be sufficiently limited to approach hermetic conditions. Gattoni (*5*) reported that large underground "silos" used for storing dry grain in South America were effective in controlling insects and preserving the quality of the grain.

Bottomley, Christensen, and Geddes (*3, 4*) investigated the influences of oxygen concentration on mold growth and biochemical changes in stored yellow corn and concluded that nearly anaerobic conditions greatly reduce but do not entirely prevent mold development, and thus spoilage of high moisture

Corn stored in sealed bins did not deteriorate to the extent that would be expected if stored in conventional bins. Sealed storage did not prevent fermentation processes which gave rise to changes in color and odor. Odor was increased and color was darkened by high moisture contents, longer storage periods, and higher grain temperatures. Corn of 18% moisture did not change measurably in storage for 6 months, but in 1 year it darkened slightly and developed a sour odor. Corn of 23 and 27% moisture developed a sour odor in less than a month and quickly darkened in color. Sharp increases in fat acidity and in number of damaged kernels occurred toward the end of the first storage year. This corn was difficult to handle with portable grain elevators, and molded rapidly upon removal from the sealed bins. Corn stored for two seasons at 27% moisture was fed to hogs with no evidence of impaired feeding value.

grain cannot be completely prevented in hermetically sealed storage.

Barr and Coonrod (*2*) described the storage of rice in sealed bins at moisture levels of 18 to 19%. An increase in fatty acids along with the formation of an objectionable odor was coincident with the loss of germination of the stored rice. Measurements made immediately after filling and sealing the bin showed a rapid increase of carbon dioxide during the first 24 hours, and an average above 21% at the end of 48 hours. Bacteria and fungi were much more prevalent in the top 1 foot of the grain when the bin was opened after 7 months of storage.

Storage Bins

Three storage bins of gastight construction were supplied for tests by the A. O. Smith Corp., Milwaukee, Wis. The bins are constructed of steel sheets with a fused glass coating bonded to the surface. Sheets are assembled with a sealing mastic between lapped and bolted joints. Filling and discharge openings are fitted with gasketed closures which maintain sealed conditions. To avoid the danger of collapse or rupture of the structure due to pressure differences from temperature changes, withdrawal of contents, or other causes, a large plastic bag, open to the outside air, is suspended under the roof. When the pressure inside the structure is reduced, air is drawn into the bag. If the pressure increases, the bag is deflated without exchange of air in contact with the stored product. Large changes in pressure are accommodated by a relief valve which permits gas to escape or air to enter when the pressure difference reaches a predetermined amount. Structures of this design have been marketed for corn or grass silage since about 1945.

The experimental storages were erected on the Purdue University Electric Farm. The bins were typical of the 14 × 40 foot commercial silo, except that they were only about 14 feet high (Figure 1). Each bin had a capacity of about 1680 bushels. Sampling ports were provided in the discharge opening near the floor and in the side walls at levels of 2, 6, and 10 feet above the floor. The ports were fitted with pipe plugs 2 inches in diameter, which could be removed for sampling.

Tests Conducted

Observations were made on eight lots of shelled corn stored in the test bins during the period 1949 to 1952. Objectives of the tests were to determine the effectiveness of sealed storage in preventing deterioration of grain; to compare the storage conditions in sealed bins with those in conventional bins; to observe the loading and unloading of damp corn; and to explore the effect of sealed storage on the utilization of the corn for feeding or for market.

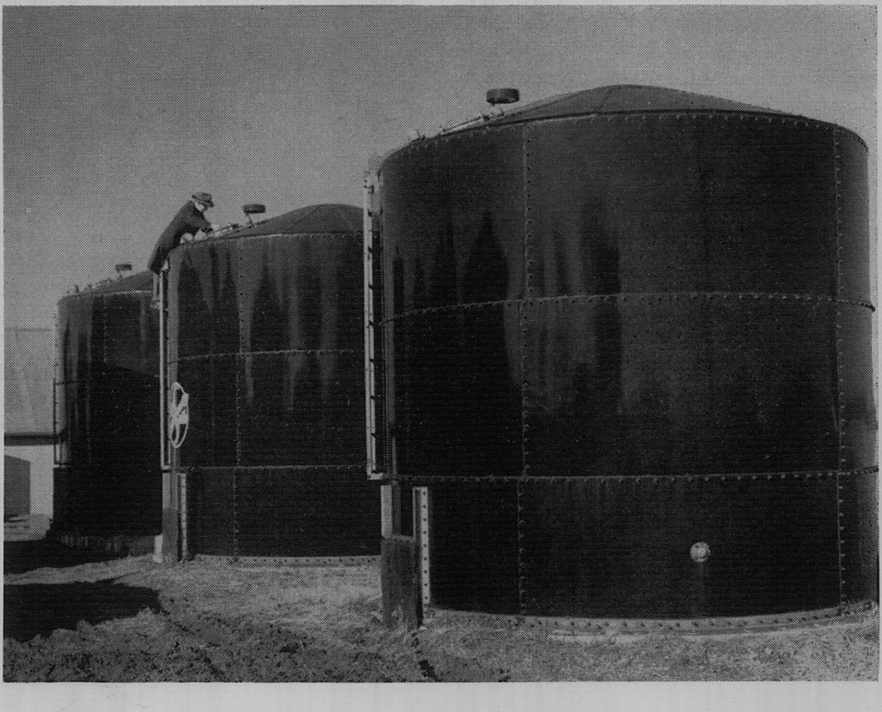
Chemical tests were made from samples drawn from the storages with a 5-foot grain probe inserted through the sampling ports. Routine chemical determinations included fat acidity (*1*) (benzene-extractable acidity expressed as milligrams of potassium hydroxide per 100 grams of dry corn), 70% ethyl alcohol–extractable nitrogen (milligrams per 100 grams of corn), free reducing sugars (milligrams of glucose per 100 grams of corn), and some measurements of starch yield and viscosity.

Commercial grade determinations were made at the beginning and end of each test. The total damage, as a grade factor, was determined at varying intervals during the storage periods.

Viability changes were measured in the last five lots of corn stored. Temperatures within the grain mass were measured by thermocouple systems installed when the bins were filled. A grid of nine thermocouples was installed in a vertical plane through the center of two bins in a north-south direction. In the third bin additional couples were installed, so that nine temperatures could also be read in a vertical east-west plane.

Erection of the bins was completed in June 1949, and a preliminary test

Figure 1. Storage bins



was started July 5. The corn used for these tests was obtained through the cooperation of the Commodity Credit Corp. and the U. S. Department of Agriculture. The corn for the first test was delivered directly from local farms, where it had been sealed in the crib under government loan. The corn as delivered was No. 2 grade and had an average moisture content of about 14.5%. As no wetter corn was readily available at that time of the year, water was added to 1600 bushels for one of the bins to increase the moisture content to about 19%. The wetting was accomplished by spraying water on the corn as it was loaded into a conditioning bin adjacent to the sealed bins. After setting overnight, the corn was transferred to the sealed bin with a mechanical elevator. The other two bins were filled with corn as shelled from the farm cribs. Tests and observations were continued on the three lots of corn until December 1, 1949.

The bins were refilled with 1949 crop corn—Indiana Hybrid 252A, grown in the Kankakee River bottom in northwest Indiana—brought directly from the field at a moisture content of about 18%. Each bin was filled about two thirds full with 1100 to 1200 bushels of corn. One bin was filled with corn at the field moisture level of 18%. The corn put in the other bins was dried artificially in the shelled form in a batch-type dryer using a maximum drying temperature of 120° F. One lot was dried to a moisture level of 13% and the other to 11%. Aside from the 18% moisture in the one lot, all the corn was No. 1 grade. The percentage of damaged kernels was less than 1%.

The two bins containing the corn stored at 11 and 13% moisture were emptied early in October 1950, and refilled with corn at a higher moisture content. Observations on the third bin containing corn at 18% moisture were continued. The 1950 crop corn was mixed varieties of Indiana hybrids, all grown in the Wabash River bottom near Lafayette, Ind. It was harvested with a conventional picker, hauled in the ear to a local elevator where it was shelled, and delivered to the experimental bins the following day. The kernel moisture content of the corn averaged 27% at harvest. About 1200 bushels were loaded directly into the airtight bin as delivered. The bin was sealed the following morning, about 48 hours after the corn was picked.

About half of the corn of 27% moisture content was ventilated with unheated air for 48 hours to lower the moisture content to 23%. However, air conditions were unfavorable for drying without heat, and mold growth started the second day. Heat was added and drying was continued another 20 hours with air temperatures up to 124° F. The corn was conditioned in a drying bin located adjacent to the test bins and was transferred to the sealed storage at an average moisture of 23%. Seven days elapsed between the time the corn was picked and the time this bin was sealed.

Storage observations were continued on the three lots at 18, 23, and 27% moisture until late summer of 1952.

An exploratory swine-feeding test was conducted between July 18 and September 26, 1952, using the corn which had been stored in the sealed bin at 27% moisture, with two lots of 15 feeding pigs each. The pigs were of mixed breeds but were sorted by the Purdue animal husbandrymen into lots as nearly alike as possible. The two lots were fed in nearly identical pasture lots equipped with the same type of feeders, waterers, and shade. The ration consisted of shelled corn, 40% commercial supplement, bone meal, and mineralized salt, all fed in self-feeders. One lot received the high moisture corn from the sealed silo and the other lot was fed dry commercial corn stored in the conventional manner. The dry corn was an Indiana hybrid of different variety and grown in a different year on a different soil type than the wet corn.

The wet corn was removed from the bin with a 6-inch auger elevator which was inserted through a small resealable hatch in the emptying door at the bottom of the bin. The corn was removed and placed in the self-feeders twice weekly. An effort was made to place in the feeders only the quantity of corn which would be consumed between feeding days.

Results

Fat Acidity. The first three storage tests were exploratory and were conducted during the summer while awaiting the harvest of a new crop in the fall. Complete chemical determinations were made during the first 8 weeks only, and were limited to the one lot of corn of 18.8% moisture, which had been artificially wetted. During this period, the fat acidity values of the probe samples showed a slowly increasing trend. The other chemical measures showed no consistent change. The samples for the final values of fat acidity and damage reported in Table I were taken from the center of the bin where deterioration appeared to be greatest, in order to explore the maximum extent of damage that occurred. The final values reported do not represent the average for the lot.

The three lots of corn put in the bins in December 1949 and early January 1950 had initial fat acidity values that ranged between 20 and 27. The corn stored at 11 and 13% moisture content showed no increase in fat acidity during the 10-month storage period. During the same period corn of 18% moisture increased about 10 units. The test with 18% corn was continued two more years.

The two lots of 1950 corn with moisture levels of 27 and 23% had initial fat acidity values of 47 and 63, respectively. These values, relatively high initially, steadily rose to just under 100 during the first 6 months (Figures 2 and 3). The next 6 months, during the warmest part of the year, the fat acidity increased at a more rapid rate and reached averages of near 200 for both lots. The change from there to the end of the test period was small. The rate of change in the corn of 23% moisture was only slightly less than that in the corn of 27% moisture content. The initial deterioration that took place in the process of conditioning the corn to the 23% moisture level probably influenced the results.

The corn of 18% moisture showed no marked rise in fat acidity until the summer of the second storage year (Figure 4). Contrary to the results with 23 and 27% corn, the increase was more rapid in the top sample of corn of 18% moisture content. There was less volume of the corn of 18% moisture content and its samples were drawn nearer the corn surface. This may account for at least part of the greater increase in fat acidity in the top of this lot.

Table I. Summary of Shelled Corn Storage Tests in Sealed Bins

Storage begun	7–5–49	7–6–49	7–8–49	1–10–50	12–20–49	12–19–49	11–2–50	10–25–50
Storage ended	12–1–49	12–1–49	12–1–49	10–3–50	10–3–50	9–1–52	10–1–52	11–1–52
No. of bushels	1680	1600	1570	1075	1112	1220	1184	1252
Moisture content, %	14.6	14.3	18.8	11	13	18	23	27
Fat acidity[a]								
Initial	39	37	34	23	20	27	63	47
Final	26[b]	44[b]	157[b]	20	20	170	170	192
Commercial damage, %								
Initial	2.6	3.2	1.0	0.6	0.6	0.6	1.3	Trace
Final	2.1[b]	6.1[b]	85[b]	0.6	0.6	11.0	85	98

[a] Fat acidity expressed as mg. potassium hydroxide per 100 grams dry corn.
[b] Final samples taken from center of bin where deterioration appeared to be greatest.

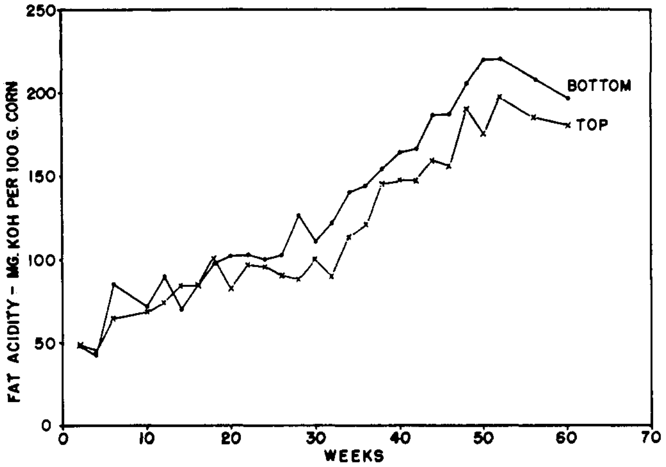


Figure 2. Changes in free fatty acids during sealed storage of corn of 27% moisture

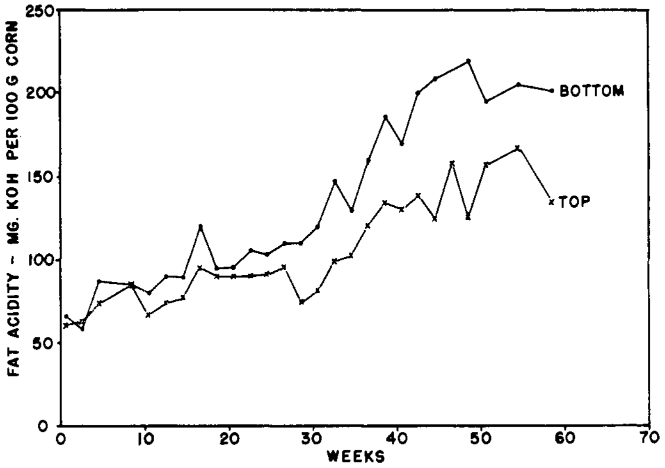


Figure 3. Changes in free fatty acids during sealed storage of corn of 23% moisture

The other chemical determinations made in these tests showed no consistent change through the storage period.

Viability. Germination data were obtained in part of the tests beginning with the final samples from the corn of 11 and 13% moisture content stored for 10 months. At this moisture level there was apparently little loss of viability, since the final samples germinated 90 and 85%, respectively. The corn of 18% moisture retained a viability of only 1% after 10 months and none after one year of storage. This much greater loss of viability than experienced with grain stored in air is due, perhaps, to the suffocation of the grain at the low oxygen levels developed in the bins. In the corn of 23% moisture viability was reduced from 65 to 25% during the first month of storage and was 0 at the end of about 3 months. The corn of 27% moisture lost all viability within 6 weeks.

Commercial Grade. The percentage of damaged kernels varied considerably throughout the test. Some of the determinations in earlier tests were made on wet samples, which showed more damaged kernels than similar samples after drying. In general, the increase in kernel damage lagged behind the increase in fat acidity, and none of the lots (except that artificially wetted) showed extensive damage during the first storage year.

The odor which developed in the corn was probably the most significant factor affecting the commercial grade of the stored corn. A sour fermentation odor developed in all lots except those stored below 14.5% moisture content. In the corn of 18% moisture, the odor was not noticeable until June following start of the test in December. The sour odor developed in corn of 23 and 27% moisture within 2 to 4 weeks after bins were filled and was more pronounced than in the 18% corn. The odor was not so intense after the corn was artificially dried, but persisted in sufficient quantity to be graded "sour" according to commercial grading standards.

The corn also changed noticeably in appearance in all lots stored above 14.5% moisture. The first noticeable change was deepening of the yellow color, followed by a slow darkening which eventually produced almost 100% mahogany or brown kernels in the corn of 27% moisture. Many of the kernels in the corn above 18% moisture softened in structure to the extent that they could be mashed easily between the thumb and forefinger. After drying, these kernels pulverized when handled and produced considerable powdered starch. Corn stored for two seasons at 27% moisture content appeared, smelled, and felt much like the corn kernels in corn silage stored in a conventional silo.

No insects were found in any of the sealed bins. There was no measurable change in total moisture content during storage and no great loss in dry weight in any of the lots stored. Samples from all of the lots above 14.5% moisture became covered with a white powdery mold within 24 hours when exposed to air at room temperature.

Grain Temperature. There was no evidence of spontaneous heating in any of the lots except during the first few hours after the bins were sealed. After the initial oxygen supply in the storage was depleted, temperatures within the grain mass followed those observed in conventional storages of similar size and shape. The grain temperature changes lagged about 2 months behind the outside temperature changes. The lowest temperatures in the grain usually occurred in the late winter and were between 33° and 40° F. The highest temperature occurred in late summer or early fall and ranged from 70° to 80° F.

Differences in temperature within the grain in the bin gave rise to moisture transfer and accumulation. Most noticeable was the transfer of moisture from the grain near the warm south wall to the colder north wall. When the bins were opened, the wetter lots showed free water on the corn 3 to 6 inches from the north wall, while corn within 3 inches of the south wall had a moisture content at least 5% less than the average of the bin.

Handling

The corn with moisture levels of 19% and below was handled with conventional auger and chain-flight elevators with little difficulty. The corn of 18 to

Figure 4. Changes in free fatty acids during sealed storage of corn of 18% moisture

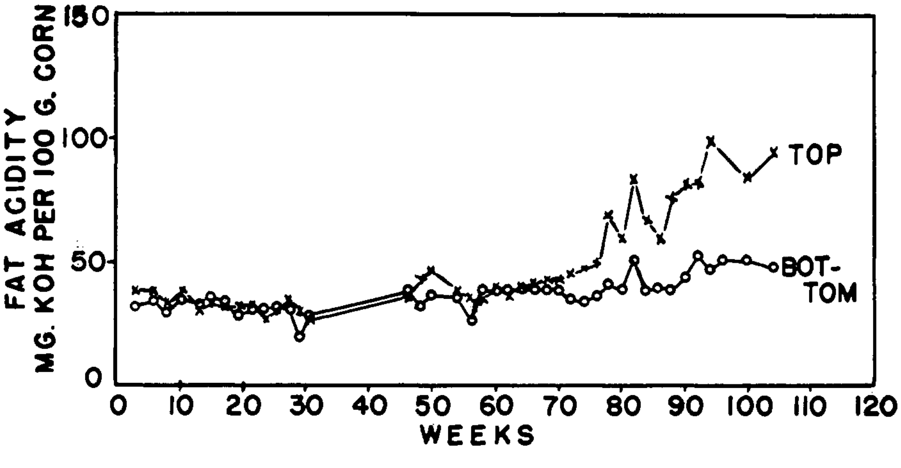


Table II. Summary of Swine Feeding Test

	Lot 1, Fed 27% Moisture Corn Stored in Sealed Bins	*Lot 2, Fed 13% Moisture Corn Stored in Conventional Bins*
Pigs per lot	15	15
Av. initial wt., lb.	85.9	86.7
Av. final wt., lb.	191.1	188.4
Av. daily gain, lb.	1.50	1.45
Av. daily feed, lb.		
Corn	5.06	4.52
Supplement, 40% protein	0.47	0.73
Feed per 100-lb. gains, lb.	368	362

19% moisture did not flow readily and the capacity of the auger elevator was reduced somewhat below that when handling dry corn.

The corn at 23 and 27% moisture was very difficult to handle with conventional equipment. It became so packed in storage that more than one man was required to insert a 5-foot sampling probe. The corn could not be moved with a grain scoop without first loosening it with a pick or probe. When unloading from the bottom of the bin, the corn bridged over the intake to the auger elevator and had to be probed loose.

The corn from the last three lots was dried in an experimental batch dryer over a 2- to 3-week period in the late summer. Although the bins were opened frequently to load the dryer, the undisturbed corn remaining in the bins did not heat or show any noticeable increase in mold growth.

Feeding Tests

Hogs fed corn of 27% moisture stored in the sealed bin for two seasons remained healthy and made normal weight gains during a 10-week test period (Table II). Compared to the lot which received dry corn, those fed wet corn gained slightly more, consumed 12% more corn, but ate 36% less protein supplement. The cost per 100 pounds gain at 1952 prices was $12.63 and $12.18 for the lots receiving dry and wet corn, respectively. Nutritionally, the large difference in the consumption of protein supplement may be significant enough to warrant further study.

The wet corn fed was brown in color and that from some parts of the bin was somewhat moldy. Part of the time the corn placed in the self-feeders became completely covered with white mold within 24 hours. At other times, little additional mold growth appeared during the 3 to 4 days between times of filling the feeders. The amount of molding in the feeders was greater during wet weather. There was some caking of corn on the sides of the self-feeder, but in general no great difficulty was encountered in self-feeding the wet corn.

Whether or not hogs do well on wet corn will depend on the extent of damage, the type of mold present, and perhaps other factors.

Acknowledgment

The authors acknowledge the assistance of C. M. Vestal, who directed the feeding tests, and of F. E.Robbins, a designated federal grain inspector, who classified the corn samples according to grade.

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WINE PRODUCTION

Determination of Free and Total Sulfur Dioxide in White Table Wines

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THE CONCENTRATION OF SULFUR DIOXIDE in wine and its distribution in free sulfurous acid, bisulfite ions, and sulfite addition products determine the stability and palatability of wine. Of the many methods available for the determination of sulfur dioxide in wine (*5, 6, 12–14, 19, 21*), the direct iodine titration procedures and the colorimetric fuchsin procedures have been more frequently suggested for the determination of "free" and "bound" sulfur dioxide. The chemistry of sulfite addition products present in wine is still unknown, although there is evidence that acetaldehyde and glucose addition products are present (*5, 6, 11–14, 19, 21, 22, 28*). Deibner and Bénard (*7*) suggested recently that the acetaldehyde-sulfurous acid (α-hydroxyalcoyl sulfuric acid) could be separated by distillation, but found that dilution affected the stability of the compound more than any possible decomposition during distillation. The conditions affecting the equilibrium between free and bound sulfur dioxide and the rate of combination, liberation, and recombination have not been investigated extensively, although it is recognized that pH, temperature, alcohol concentration, concentration of glucose and acetaldehyde, and concentration, type, and method of adding sulfur di-