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MICROBIOLOGICAL AND CHEMICAL STUDIES OF SWEET POTATO VINE SILAGE[1]

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This study was conducted as part of an investigation on the preservation of sweet potato vines by ensiling. Fresh vines, which yield an average of 10 to 20 tons per acre, are readily eaten by livestock, and it was believed they would be valuable as roughage for winter feeding if properly preserved. A review of the literature showed that Bennett and Gieger *(3)* ensiled sweet potato vines and mixtures of vines, phosphoric acid, and molasses in sealed fruit jars. These workers concluded that silage could not be made from vines alone but that good silage was made when phosphoric acid or molasses was added. In our investigation, data were sought from vines and mixtures of vines, tubers, and molasses by determining the progressive changes which occur in the microbial flora and chemical composition during the fermentation period. The work was conducted during 1941-1943 at the North Carolina State College with the cooperation of several departments (Agricultural Engineering, Animal Industry, and Horticulture) as well as the Agricultural Research Service of the U. S. Department of Agriculture. The results from feeding tests with sweet potato vines ensiled in silos will be reported separately by the Department of Animal Industry, North Carolina State College.

MATERIALS AND METHODS

Treatment of materials. Green, succulent vines and leaves (hereafter referred to as sweet potato vines) of the Puerto Rico variety of sweet potatoes were removed from the plants by hand or with a mechanical vine harvester, previously described by Hendrix *(9)*. Sweet potato roots were dug from the fields where the vines were harvested. Feed-grade molasses was used to increase the carbohydrate content of some silage. The silage mixtures were prepared by running the materials through a conventional silage cutter, which chopped the vines into approximately 1- to 1.5-in. lengths and the potatoes into irregular-shaped pieces about 0.25-0.75 in. in size.

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The composition of the barrel materials prepared each year is given in Table 1. Replicate 50-gal. wooden barrels each were filled with about 350 lb. of a mixture, and the material was packed firmly by treading. Thermocouples were placed in duplicate barrels of each treatment at about 10 to 12 in. from the top and bottom ends for the purpose of recording temperatures. After being

TABLE 1

Composition of materials ensiled in barrels for study of progressive microbial and chemical changes

Treatment	Composition
A	Sweet potato vines, 100%
B	Sweet potato vines, 85% and sweet potato tubers, 15%
C	Sweet potato vines, 83.5%, sweet potato tubers, 13.5%, and sugar cane molasses, 3%

Each barrel contained approximately 350 lb. of material. Barrels were filled during 3-year period as follows: 1941, on October 23; 1942, October 24; and 1943, October 12.

filled, the barrels were headed and stored under a roof shelter. Silages of the same composition made at the same time in 1-gal. glass jars with screw-type lids were used in this comparative study. However, the bacteriological and chemical studies reported here were conducted mainly on barreled silage. Samples of silage for analysis were obtained from duplicate barrels of each treatment by boring a 2-in. hole into the side of the barrel. A new hole was made at each sampling, after which it was closed with a stopper. One-pound samples were removed aseptically by a wire hook to sterile containers for bacteriological examination. Two-pound samples were similarly removed for chemical analysis. In 1941 no attempt was made to displace with gas the air in the void caused by removing the sample. However, in 1942 and 1943 the air was displaced with carbon dioxide to eliminate the effect of oxygen on the flora. Samples were obtained over a period of 69 days in 1941, 202 days in 1942, and 39 days in 1943. During 1941, 36 barrels of Treatment C silage were prepared for a statistical study of sampling methods to determine the effect on the flora, population of microorganisms, and chemical changes in barrels sampled repeatedly, as compared with previously unopened barrels. For this study the samples were taken at well-spaced distances from the barrels, which were opened repeatedly. Samples from previously unopened barrels were taken from the middle section. Silage made in glass jars was similarly studied. Only one sampling was made from each glass jar.

Bacteriological and chemical examination of silage. Media and other conditions for the cultivation and enumeration of the microorganisms have been described by Etchells and Jones *(8)*. Acid-forming and other mesophilic aerobic bacteria were grown on nutritive caseinate agar (Difco).[6] Coliform bacteria, primarily species of *Aerobacter,* were grown on brilliant green lactose-bile agar (Difco), and yeasts and molds were grown on acidified dextrose agar. The

[6] The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

latter medium consisted of ordinary dextrose agar (Difco formula) to which 5 ml. of sterile 5% tartaric acid was added just prior to pouring the plates. Viable cell counts were made on these media from serial dilutions of silage washings (50 g. of sample shaken in 450 ml. of sterile water). Microscopic examination of the ensiled material was made by use of smears prepared from the silage washings by the method of Wang *(11)*, a modification of the Breed *(4)* technique. Microscopic counts of Gram-positive cells were made for large and small bacilli, large and small cocci, and yeasts.

Juice expressed from about 2 lb. of silage by a Carver hydraulic laboratory press was used for chemical analyses. pH was measured potentiometrically with a glass electrode. Sugars, as reducing sugar, were determined by the method of Shaffer and Somogyi *(10)*. Total acidity, expressed as lactic acid, was determined by titrating 10 g. of expressed juice (heated to a boil) with 0.1 *N* NaOH. Moisture was determined by drying in vacuo at 70° C.

RESULTS AND DISCUSSION

The initial composition of the material varied from year to year; yet the population trends of microorganisms and the chemical changes were the same within silage treatments. For this reason most of the data relative to progressive microbiological and chemical changes are presented graphically and represent average values for 3 years, except mold populations, which are for 2 years. The population trends for lactic acid-forming bacteria, *Aerobacter* sp., yeasts, and molds have been combined for all treatments for the 3 years and are shown in Figure 1.

Acid-forming bacteria. Lactic acid-forming bacteria were present in the ensiled material and comprised the majority of bacteria which grew on nutritive caseinate agar. This group predominated throughout the sampling periods. The average population of acid-forming bacteria varied initially from less than 10 thousand to 29 million per gram in the freshly barreled mixtures and increased rapidly during the first days of the fermentation. Maximum populations of about 150 to 600 million per gram were reached in about 5 days. After this there was a general decline in numbers throughout the sampling period. The majority of acid-forming bacteria were identified as *Lactobacillus plantarum*. This microorganism was likewise found by Allen *et al. (2)* to predominate in grass silage and to reach a population of 1 billion per gram after about 17 days. They observed the presence on the fresh grass of a mixed flora including lactobacilli, coliform bacteria, spore-forming bacteria, molds, and yeasts. Among these, *Bacillus subtilis* and *B. megaterium* also were found to increase greatly in numbers at the peak of the fermentation. Allen and coworkers also noted that thermophilic organisms identified as strains of *B. subtilis* increased in numbers with a rise in the temperature of the silage. Cunningham and Smith *(6)* observed strains of *L. plantarum* as well as *L. brevis* in silage made by the addition of mineral acids to plant materials. These workers reported that the flora consisted mainly of lactic acid–forming bacteria.

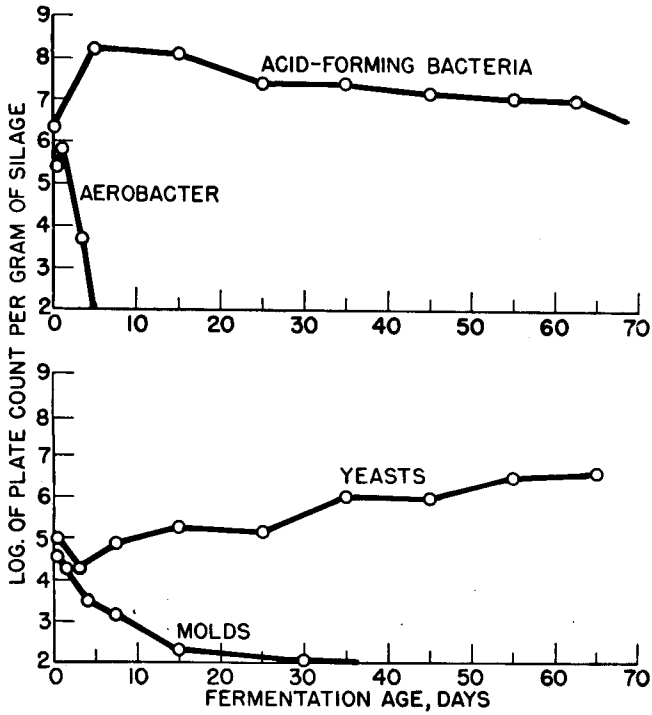

FIG. 1. Population trends of viable microorganisms in barreled silage.

Coliform bacteria. Variable numbers of coliform bacteria are present on the sweet potato vines, potatoes, and molasses. Figure 1 shows that the population of this group of organisms increased substantially during the first day and declined rapidly thereafter. The data suggest that the formation of lactic acid in the silage may be the limiting factor in the growth and survival of coliform bacteria. Etchells and coworkers *(7)* have shown that coliform bacteria do not survive during the fermentation of certain brined and salted vegetable material in which lactic acid is formed. These workers also demonstrated that coliforms were absent from fermentations that were acidified at the outset. Apparently these organisms contribute little to the fermentation of sweet potato vine silage.

Yeasts. Viable yeasts, comprising both budding and filamentous types, were present in all silage treatments throughout the sampling period. The initial counts ranged from 2 thousand to 1 million per gram and did not exceed 15 million per gram at any sampling. During 1942, the viable yeast population was exceedingly low and did not exceed 310 thousand per gram.

Yeasts characterized as the more oxidative, film-forming types were present to the extent of about 40 thousand cells per gram in the silage when first barreled. However, they were not observed on plating media until the 20th day of the fermentation. Since they were not present on platings from silage in barrels which had not been sampled previously, it is likely that they grew only in

barreled silage following admission of oxygen through repeated samplings. Thus, in silages that are poorly packed and exposed to air these yeasts might be expected to grow and utilize part of the acid and impair the keeping quality.

Molds. The initial mold counts of the barreled silage in 1942 and 1943 were 10 thousand and 79 thousand per gram, respectively. There was a gradual decline of the population beginning with the first day, and at 30 days mold colonies were almost completely absent from plated samples. There is no indication that molds contribute favorably to the fermentation of the silage.

It is of interest that in grass silage Allen and coworkers *(1)* noted a decrease in the amount of lactic acid toward the close of their experiments, which indicated to them that this acid was being decomposed, either by other groups of bacteria or by mold which was in evidence. The near absence of mold in our silage may be due, in part, to the physical properties of the ensiled material. Sweet potato vines have excellent packing qualities and do not provide much opportunity for the invasion of air.

Microscopic examination. Direct microscopic examination of washings from silage of each of the three treatments supplemented platings during the 1941 and 1942 seasons. Included were differentiations of types of microorganisms, measurement of cell sizes, and estimation of the maximum populations averaged for all treatments. These are given in Table 2. The size and appearance of these

TABLE 2

Microorganisms in sweet potato vine silage determined by microscopic examination

Microorganism	Size	Maximum population per g. $\times 10^6$ (average all treatments)	
	(microns)	*1941*	*1942*
Rods[a]			
large	0.9 × 2.7 to 4.0 0.9 × 6.3 to 15.0	1155	103
small	0.68 × 1.3 to 2.24	555	319
Cocci[a]			
large	1.4 to 1.8	63	31[c]
small	0.9 to 1.1	40	31
Yeasts			
fermentative[b]	2.3 to 3.6 × 3.6 to 4.5	72	6
oxidative	1.8 × 14.4 to 2.7 × 12.6	13	0

[a] Data are for Gram-positive organisms.
[b] Isolates identified were *Zygosaccharomyces* sp.
[c] Count of large and small cocci.

microorganisms remained uniform in the three silage treatments for these years. It is noted that the largest populations for each type of microorganism were obtained during 1941, a year during which the fermentation temperature exceeded all others. The differences are of such magnitude as to warrant consideration and, since they are greatest among the acid-forming organisms, they are treated in the forthcoming section on acidity. The combined average populations

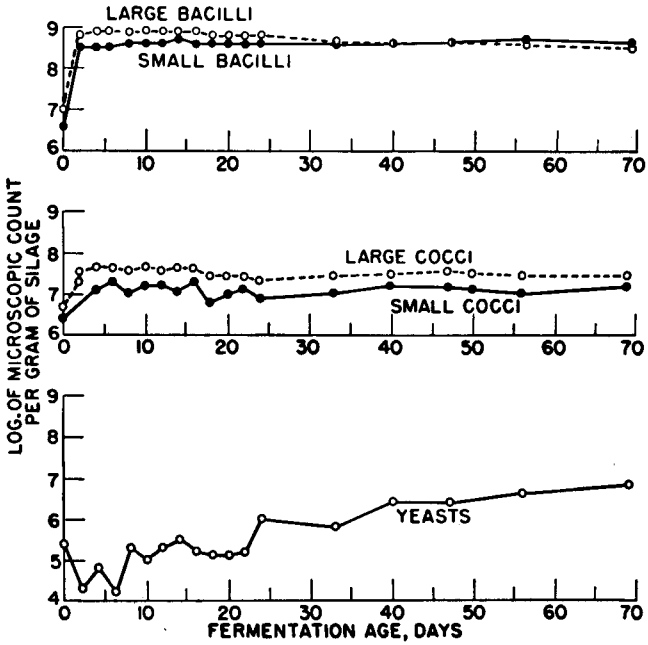

FIG. 2. Population trends of Gram-positive bacteria and of yeasts in barreled sweet potato vine silage.

of Gram-positive large and small bacilli, large and small cocci, and yeasts for 1941 are shown in Figure 2. The population trends are similar to those for viable acid-forming bacteria and yeasts. These results show that in the silage there is no reproduction of acid-forming bacteria after the initial active fermentation phase. These results also confirm the growth trend of yeasts in the silage. No attempt was made to determine the role of the different types of bacilli and cocci on the fermentation.

Chemical changes during fermentation of silage. Chemical analysis of the ensiled material shows some variation of its initial composition from year to year; however, examination shows that the chemical changes which took place during the fermentation followed the same course. For this reason progressive chemical changes, like the microbiological data, are presented graphically. The trends for pH, sugar, and acidity have each been combined for all treatments for the 3 years and are shown in Figure 3. The initial moisture contents of the vines was 78.5% for 1941, 83.7% for 1942, and 86.2% for 1943. The addition of potatoes and/or molasses lowered the initial moisture about 4.8%. The moisture content of the silage did not change appreciably during the fermentation.

pH. The initial pH of the chopped vines was 5.2 to 6.0 and showed only slight changes by the addition of potatoes or molasses to the vines. The onset of the fermentation was marked by an immediate and perceptible lowering of pH for about 15 days, after which there was no further change. The constancy of pH was additionally determined in 1942 when barrels of silage of each treatment

were examined after storage for 202 days. These results indicate that the most active stage of the acid fermentation was during about the first 15 days.

Sugars. Sugars in the press juices from the vines and potatoes occurred as reducing sugars; therefore, sugars added by molasses were determined as reducing sugars. The initial reducing-sugar content of juices from Treatments A and B for all years ranged from 1.92 to 3.58% and averaged 2.33%. The disappearance of sugar followed a similar trend for each silage treatment during the fermentation. For this reason, data from these treatments have been combined (Figure 3). Molasses increased the average sugar content of the juices from

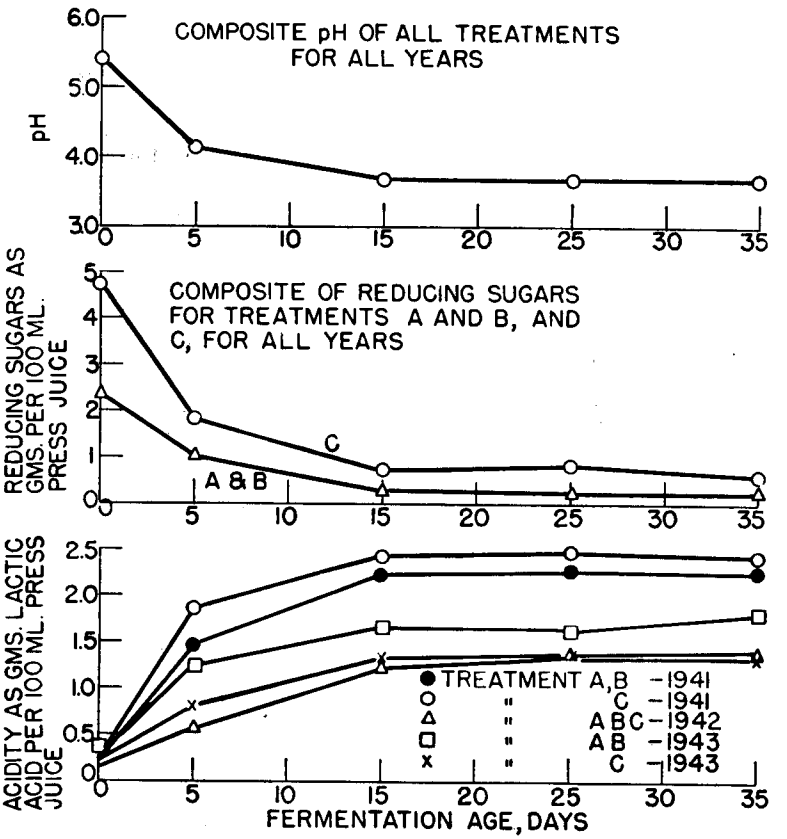

FIG. 3. Chemical changes occurring in barreled sweet potato vine silage.

silage of Treatment C to 4.75%. Data from the examination of the silage juices showed reduction of the sugar content beginning with the first day and continuing for about 15 days. There was no substantial further utilization of sugar beyond 35 days, as it was found that the residual sugar in Treatments A and B in 1942 was 0.15% and in Treatment C it was 0.25% in silage stored for 202 days.

Acidity. The data show that the formation of acid follows trends similar to those for pH, the disappearance of sugar, and also the population trend for

acid-forming bacteria. Although the initial acidity of the juice from all treatments of silage for the 3 years was relatively uniform, there were differences in the rate and amount of acid formed during the fermentation. All treatments attained maximum acidity in about 15 days, during the period when populations of viable bacteria were at a maximum. Temperature appears to influence the rate of acid formation and probably that of the activity of the acid-forming bacteria. Temperature data given in Table 3 show the average daily temperature

TABLE 3

Temperature of silage at sampling intervals

1941 season			1942 season			1943 season		
Date	Sampling interval	Silage temp.	Date	Sampling interval	Silage temp.	Date	Sampling interval	Silage temp.
	(days)	*(° F.)*		*(days)*	*(° F.)*		*(days)*	*(° F.)*
October			October			October		
23	0	76	24	0	59	12	0	—
25	2	75	25	1	—	13	1	67
27	4	66	26	2	—	14	2	68
29	6	71	27	3	59	15	3	70
31	8	64	28	4	53	16	4	71
			30	6	56	18	6	62
November						20	8	60
2	10	65	November			22	10	62
4	12	70	3	10	59	26	14	60
6	14	65	7	14	54			
8	16	64	13	20	53	November		
10	18	57	23	30	61	1	20	61
12	20	50				11	30	51
14	22	50				20	39	52
16	24	56	1943					
25	33	—	January					
December			8	77	38			
2	40	—	May					
9	47	51	13	202	—			
18	56	43						
31	69	47						
Av. temp. during first 20 days		65.7			56			58

of the silage at sampling times for the first 20 days of the fermentation to be 65.7° F. in 1941, 58° F. in 1943, and 56° F. in 1942. The rate of acid formation was likewise greatest in 1941, next in 1943, and lowest in 1942. There was no evidence of heating of the silage, and this may account for the prolonged period of high viable bacterial populations throughout the experiments. The expected relationship between the amount of acid formed and the initial sugar content of the silage mixtures was found only during 1941 and 1943 for the different silage treatments. During 1942, there was almost no effect on the total acids formed by the addition of molasses to the silage mixtures. This lack of difference may have been the effect of low fermentation temperatures. The disappearance of sugar in the 1942 silages, accompanied by low acid production, suggests that the low

temperature perhaps favored the fermentative activity of yeasts or other sugar–utilizing but nonacid–forming organisms. These combined data indicate, however, that within the limits of the conditions of the experiments, acid–type fermentations occurred in all treatments with the formation of lactic acid mainly, which resulted in the preservation of the ensiled materials. The odor, color, and physical characteristics of the silage from all of these treatments were typical of good quality silage.

Sweet potato vines ensiled with urea. During the 1943 season, a mixture of 99% sweet potato vines and 1.0% urea was ensiled in barrels to determine the effect of this nitrogen compound on the bacterial flora and chemical composition of the silage. The bacteriological data showed that urea did not exert a statistically significant difference with respect to time trends, or populations, except that this treatment supported the survival of *Aerobacter* sp. in a significantly higher number and for a longer period than did the three other treatments. Data showing populations and survival of *Aerobacter* sp. in these silages are given in Table 4. It will be seen that *Aerobacter* sp. survived for 8 days in silage composed of vines and urea, whereas they failed to survive more than 3 days

TABLE 4

The average numbers of coliform bacteria at different times on vines + urea and other treatments in 1943

Age of silage	Thousands per g. of silage	
	Vines + urea	Other treatments
(days)		
0	31.0	23.4
1	570.0	141.9
2	175.0	18.8
3	4.1	0.2
4	0.7	0.0
6	7.1	0.0
8	0.8	0.0
10-30	0.0	0.0

in the other treatments during this particular year. A comparison of the chemical data for silage composed of vines alone and that of vines and urea revealed similar trends for pH, acidity, and the disappearance of sugar. There are no important differences for these values. The silage containing urea had good odor, color, and texture throughout the sampling period.

The beneficial results of adding urea to silage have been reported by Cullison *(5)*. He reported that the prolonged fermentation period, which occurs when silage is made from sweet sorghum alone, was eliminated by the addition of 10 lb. of urea per ton of silage material. The resulting silage was superior in carotene content, palatability, and general feeding value to that made from sweet sorghum without urea.

Although the present study did not show a beneficial effect for urea on the fermentation of sweet potato vine silage, it could have the other values cited by Cullison. The present widespread use and recognized value of urea with

roughage feed for multistomach animals certainly suggest that it might be a useful additive to silage.

Analysis of data on experimental techniques. Several questions on experimental techniques were apparent at the outset of this study, and some of them were tested along with the major objectives. It was desired to know the effect of different sampling methods on the flora, population of microorganisms, and the chemical changes in barrels sampled repeatedly, as compared with previously unopened barrels. There was also the question of the effect of the type of container on the flora, particularly glass containers, which were used in other investigations (Bennett and Gieger, *3*). Some of these data were analyzed statistically to determine the more precise effect of these variables on the microbial populations and growth trends.

Effect of different sampling methods. Two methods were studied in 1941 in following the course of the fermentation: *(a)* taking samples from the same barrels on successive dates and *(b)* taking samples from new barrels opened each time. The bacteriological results for the two methods did not differ significantly with respect to time trends, or mean or peak numbers of acid-forming bacteria, but yeast counts were decidedly lower when new barrels were opened each time. These differences are shown graphically in Figure 4. It was assumed

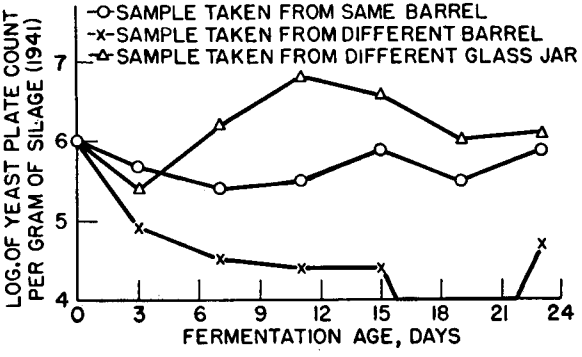

FIG. 4. Population trends of viable yeasts in silage taken each time from the same barrel versus a different barrel and from a different glass jar.

that the fundamental difference between the two methods was one of aeration, and that this was a more important factor in yeast development than in acid-forming bacteria.

Effect of type of containers. Wooden barrels were compared with glass jars as suitable containers for such experiments when a new container was opened at each sampling date. The glass jars gave significantly higher yeast and mold counts as well as more irregular time trends than the barrels. These differences also are shown in Figure 4. The reason for these variations is not known but they may be due, in part, to temperature. The temperature of the silage in the jars was more irregular than that of the barrel silage in that it more nearly

corresponded to that of the atmosphere. The disappearance of sugar from silage in glass jars was not determined, but the pH drop and formation of acid was less than that of the barreled silage. Nevertheless, the silage in glass jars had good color, odor, and texture and appeared like that in the barrels.

Sources of experimental error. Difficulties of handling nonnormally distributed data such as result from studies with this type material (leaves, stems, and tubers) usually result in the use of approximation techniques. Nevertheless, the results are accurate enough to permit sound conclusions about the relative importance of different sources of experimental error. The relative magnitude of errors from the different sources was approximately the same for acid-forming bacteria and yeasts. Expressed as coefficients of variation they are:

Plating and counting	6%
Samples within a barrel	50%
Barrels with the same treatment	15%

In determining the most efficient technique to use, three considerations must be taken into account: *(a)* the precision desired, *(b)* the expense and effort involved in using more barrels compared to taking more samples per barrel, and *(c)* the limiting number of barrels and samples which can be handled. Table 5 shows

TABLE 5

Least significant difference (P = 0.05) in acid-forming bacteria expected from varying the number of barrels and samples per treatment (expressed as percentage of the higher treatment mean)

Samples per barrel	Barrels per treatment			
	2	4	6	8
1	90	54	42	36
2	83	42	32	28
4	69	33	25	21
8	58	27	20	17
16	50	22	17	13
32	44	19	15	12

the relative accuracy that could be expected by varying the number of barrels and samples. For example, assume a new experiment was planned in which each treatment was placed in four barrels and two samples per barrel drawn. If Treatment A had 100 million acid-forming bacteria per gram, Treatment B would have to be down to 100–42, or 58, million per gram to be significantly different.

SUMMARY AND CONCLUSIONS

Sweet potato vines and combinations of vines, tubers, and molasses were ensiled during consecutive years in glass jars and wooden barrels. There resulted under each condition an acid-type fermentation with the production of silage having good odor, color, and texture. During the most active phase of the fermentation, the population of acid-forming bacteria, which was mainly *L. plantarum,* increased rapidly from 150 to 600 million per gram of silage in

about 5 days. Although the population of acid-forming bacteria varied from year to year, their numbers were not greatly influenced by the addition of sweet potato tubers or molasses to the vines.

Chemical changes during the fermentation were evidenced by a drop in pH, a decrease in the sugar content, and the formation of lactic acid. The addition of tubers and molasses to vines increased the amount of acid formed, over vines alone, except one year when low temperatures prevailed throughout the active fermentation phase.

Analysis of data on techniques shows different sources of experimental error to be 6% for plating and sampling, 50% for samples taken repeatedly from the same barrel, and 15% for samples taken each time from different barrels.

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